[0502] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0503] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0504] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan sueh as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0505] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the moleeule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[0506] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specifie embodiment, the kits of the present invention contain a substantially isolated polypeptide eomprising an epitope which is specifieally immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody whieh does not react with the

polypeptide of interest. In another speeific embodiment, the kits of the present invention comprise two or more antibodies (monoclonal and/or polyclonal) that recognize the same and/or different sequences or regions of the polypeptide of the invention. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0507] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0508] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0509] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreaetive with polypeptide or polynueleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody.

Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0510] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0511] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0512] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

[0513] The invention further relates to antibodies which act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also included are receptor-specific antibodies which both prevent ligand binding and receptor activation. Likewise, included are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby

preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies which activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. Further included are antibodies that bind to Neutrokine-alpha and/or Neutrokine-alphaSV irrespective of whether Neutrokine-alpha or Neutrokine-alphaSV is bound to a Neutrokine-alpha Receptor. These antibodies act as Neutrokine-alpha and/or Neutrokine-alphaSV agonists as reflected in an increase in cellular proliferation in response to binding of Neutrokine-alpha and/or Neutrokine-alphaSV to a Neutrokine-alpha receptor in the presence of these antibodies. The above antibody agonists ean be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng, B. et al., Blood 92(6):1981-1988 (1998); Chen, Z. et al., Cancer Res. 58(16):3668-3678 (1998); Harrop, J.A. et al., J. Immunol. 161(4):1786-1794 (1998); Zhu, Z. et al., Cancer Res. 58(15):3209-3214 (1998); Yoon, D.Y. et al., J. Immunol. 160(7):3170-3179 (1998); Prat, M. et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard, V. et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard, J. et al., Cytokinde 9(4):233-241 (1997); Carlson, N.G. et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman, R.E. et al., Neuron 14(4):755-762 (1995); Muller, Y.A. et al., Structure 6(9):1153-1167 (1998); Bartunek, P. et al., Cytokine 8(1):14-20 (1996) (said references ineorporated by reference in their entireties).

[0514] At least fourteen murine monoclonal antibodies have been generated against Neutrokine-alpha. These monoclonal antibodies are designated: 12D6, 2E5, 9B6, 1B8, 5F4, 9A5, 10G12, 11G12, 16B4, 3D4, 16C9, 13D5, 15C10, and 12C5. Preliminary analysis of these antibodies indieates that eaeh binds Neutrokine-alpha protein in a Western blot analysis and when Neutrokine-alpha protein is bound to an ELISA plate. However, further analysis of antibodies 12D6, 2E5, 9B6, 1B8, 5F4, 9A5, 10G12, 11G12, and 16B4 indicates that only the antibodies designated 12D6, 9B6, 2E5, 10G12, 9A5, and 11G12 bind a membrane-bound form of Neutrokine-alpha. Thus, a subset of the monoclonal antibodies generated against Neutrokine-alpha have been determined to bind only the membrane-bound form of Neutrokine-alpha (i.e., this subset does not bind the soluble form of Neutrokine-alpha corresponding to amino acids 134 to 285 of SEQ ID

NO:2), which as discussed herein, is primarily limited to expression on monocytes and dendritic cells.

[0515] Antibody 9B6 has been found to bind specifically to the membrane – bound form of Neutrokine-alpha, but not to the soluble form of Neutrokine-alpha.

[0516] Epitope mapping of antibody 9B6 has indicated that this antibody binds specifically to an amino acid sequence contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2. More particularly, epitope mapping has indicated that antibody 9B6 binds specifically to a peptide comprising amino acid residues Lys-173 to Lys-188 of SEQ ID NO:2.

[0517] In contrast, antibodies 16C9 and 15C10 have been found to bind the soluble form of Neutrokine-alpha (amino aeids 134 to 285 of SEQ ID NO:2) and to inhibit Neutrokine-alpha-mediated proliferation of B cells. *See for example*, Example 10. The 15C10 antibody has also been found to inhibit binding of Neutrokine-alpha to its receptor. Epitope mapping of antibody 15C10 has indicated that this antibody binds specifieally to an amino acid sequence eontained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2. More particularly, epitope mapping has indicated that antibody 15C10 binds specifically to a peptide eomprising amino acid residues Val-227 to Asn-242 of SEQ ID NO:2. Antibody 15C10 also binds specifically to a peptide eomprising amino acid residues Phe-230 to Cys-245 of SEQ ID NO:2. It is likely that the epitope of 15C10 is conformational rather than linear and that antibody 15C10 may make specific binding contacts with amino acid residues in the full length Neutrokine-alpha protein outside of amino acid residues 223-246 of SEQ ID NO:2 as well as within amino aeid residues 223-246.

[0518] Furthermore, eompetitive binding studies have shown that antibodies 3D4 and 15C10 bind similar or identical epitopes (see Example 15).

[0519] As deseribed above, anti-Neutrokine-alpha monoclonal antibodies have been prepared. Hybridomas producing the antibodies referred to as 9B6 and 15C10 were deposited with the ATCC loeated at 10801 University Boulevard, Manassas, Virginia 20110-2209, on January 27, 2000 and were assigned deposit accession numbers PTA-1158 and PTA-1159, respeetively. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0520] NS0 cell lines engineered to secrete chimeric forms of antbodies 3D4 an 15C10 were deposited with the ATCC located at 10801 University Boulevard, Manassas, Virginia 20110-2209, at on October 24, 2001 and were assigned deposit accession numbers PTA-3795 and PTA-3794, respeetively. Chimeric antibodies 3D4 and 15C10 contain murine variable regions and human constant (IgG1 and kappa) regions. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0521] In one embodiment, the antibodies of the invention have one or more of the same biological characteristics as one or more of the antibodies secreted by the deposited eell lines (ATCC accession numbers PTA-1158, PTA-1159, PTA-3795 and PTA-3794). By "biological characteristics" is meant, the in vitro or in vivo activities or properties of the antibodies, such as, for example, the ability to bind to Neutrokine-alpha (e.g., the polypeptide of SEQ ID NO:2, the mature form of Neutrokine-alpha, the membrane-bound form of Neutrokine-alpha, the soluble form of Neutrokine-alpha (amino acids 134 to 285 of SEQ ID NO:2), and an antigenic and/or epitope region of Neutrokine-alpha), the ability to substantially block Neutrokine-alpha/Neutrokine-alpha receptor binding, or the ability to block Neutrokine-alpha mediated biological activity (e.g., stimulation of B cell proliferation and immunoglobulin production). Optionally, the antibodies of the invention will bind to the same epitope as at least one of the antibodies specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

[0522] Thus, in one embodiment, the invention provides antibodies that specifically bind the membrane-bound form of Neutrokine-alpha and do not bind the soluble form of Neutrokine-alpha. These antibodies have uses which include, but are not limited to, as diagnostic probes for identifying and/or isolating monocyte lineages expressing the membrane bound form of Neutrokine-alpha. For example, the expression of the membrane bound form of Neutrokine-alpha is elevated on activated monocytes, and accordingly, antibodies encompassed by the invention may be used to detect and/or quantitate levels of activated monocytes. Additionally, antibodies that only bind the membrane bound form of Neutrokine-alpha may be used to target toxins to neoplastic, preneoplastic, and/or other cells that express the membrane bound form of Neutrokine-alpha (e.g., monocytes and dendritic eells).

[0523] In another embodiment, antibodies of the invention specifically bind only the soluble form of Neutrokine-alpha (amino acids 134 to 285 of SEQ ID NO:2). These antibodies have uses which include, but are not limited to, uses such as diagnostic probes for assaying soluble Neutrokine-alpha in biological samples, and as therapeutic agents that target toxins to cells expressing Neutrokine-alpha receptors (e.g., B cells), and/or to reduce or block in vitro or in vivo Neutrokine-alpha mediated biological acitivty (e.g., stimulation of B cell proliferation and/or immunoglobulin production).

[0524] The invention also provides for antibodies that specifically bind both the membrane-bound and soluble form of Neutrokine-alpha.

[0525] As described above, the invention encompasses antibodies that inhibit or reduce the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind Neutrokine-alpha receptor and/or Neutrokine-alphaSV receptor in vitro and/or in vivo. In a specific embodiment, antibodies of the invention inhibit or reduce the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind Neutrokine-alpha receptor and/or Neutrokine-alphaSV receptor in vitro. In another nonexclusive specific embodiment, antibodies of the invention inhibit or reduce the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind bind Neutrokine-alpha receptor and/or Neutrokine-alphaSV receptor in vivo. Such inhibition can be assayed using techniques described herein or otherwise known in the art.

[0526] The invention also encompasses, antibodies that bind specifically to Neutrokine-alpha and/or Neutrokine-alphaSV, but do not inhibit the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind Neutrokine-alpha receptor and/or Neutrokine-alphaSV receptor in vitro and/or in vivo. In a specific embodiment, antibodies of the invention do not inhibit or reduce the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind Neutrokine-alpha reeeptor and/or Neutrokine-alphaSV receptor in vitro. In another nonexclusive specific embodiment, antibodies of the invention do not inhibit or reduee the ability of Neutrokine-alpha and/or Neutrokine-alphaSV to bind Neutrokine-alpha receptor and/or Neutrokine-alphaSV receptor in vivo.

[0527] As deseribed above, the invention encompasses antibodies that inhibit or reduee a Neutrokine-alpha and/or Neutrokine-alphaSV-mediated biological activity in vitro and/or in vivo. In a speeific embodiment, antibodies of the invention inhibit or reduce Neutrokine-alpha- and/or Neutrokine-alphaSV-mediated B cell proliferation in vitro. Such inhibition ean be assayed by routinely modifying B eell proliferation assays

described herein or otherwise known in the art. In another nonexclusive specific embodiment, antibodies of the invention inhibit or reduce Neutrokine-alpha- and/or Neutrokine-alphaSV-mediated B cell proliferation in vivo. In a specific embodiment, the antibody of the invention is 15C10, or a humanized form thereof. In another preferred specific embodiment, the antibody is 16C9, or a humanized form thereof. Thus, in specific embodiments of the invention, a 16C9 and/or 15C10 antibody, or humanized forms thereof, are used to bind soluble Neutrokine-alpha and/or Neutrokine-alphaSV and/or agonists and/or antagonists thereof and thereby inhibit (either partially or completely) B cell proliferation.

[0528] Alternatively, the invention also encompasses, antibodies that bind specifically to a Neutrokine-alpha and/or Neutrokine-alphaSV, but do not inhibit or reduce a Neutrokine-alpha and/or Neutrokine-alphaSV-mediated biological activity in vitro and/or in vivo (e.g., stimulation of B cell proliferation). In a specific embodiment, antibodies of the invention do not inhibit or reduce a Neutrokine-alpha and/or Neutrokine-alphaSV-mediated biological activity in vitro. In another non-exclusive embodiment, antibodies of the invention do not inhibit or reduce a Neutrokine-alpha and/or Neutrokine-alphaSV mediated biological activity in vivo. In a specific embodiment, the antibody of the invention is 9B6, or a humanized form thereof.

[0529] As described above, the invention encompasses antibodies that specifically bind to the same epitope as at least one of the antibodies specifically referred to herein, in vitro and/or in vivo.

[0530] In a specific embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequenee contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Lys-173 to Lys-188 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Lys-173 to Lys-188 of SEQ ID NO:2, in vivo.

[0531] In an additional specific embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Val-227 to Asn-242 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Val-227 to Asn-242 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Phe-230 to Cys-245 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Phe-230 to Cys-245 of SEQ ID NO:2, in vivo.

[0532] The invention also provides antibodies that competitively inhibit the binding of the 9B6 monoclonal antibody produced by the hybridoma deposited as PTA-1159 to a polypeptide of the invention, preferably the polypeptide of SEQ ID NO:2, more preferable to a polypeptide having the amino acid sequence of residues Ser-171 to Phe-194 of SEQ ID NO:2. Competitive inhibition can be determined by any method known in the art, for example, using the competitive binding assays described herein. In preferred embodiments, the antibody competitively inhibits the binding of 9B6 monoclonal antibody by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, to the polypeptide of SEQ ID NO:2, or more preferable to a polypeptide having the amino acid sequence of residues Ser-171 to Phe-194 of SEQ ID NO:2.

[0533] The invention also provides antibodies that competitively inhibit the binding of the 15C10 monoclonal antibody produced by the hybridoma deposited as PTA-1158 to a polypeptide of the invention, preferably the polypeptide of SEQ ID NO:2, more preferable to a polypeptide having the amino acid sequence of residues Glu-223 to Tyr-246 of SEQ ID NO:2. In preferred embodiments, the antibody competitively inhibits the binding of 15C10 monoclonal antibody by at least 95%, at least 90%, at least 85%, at least 80%, at

least 75%, at least 70%, at least 60%, at least 50%, to the polypeptide of SEQ ID NO:2, or more preferable to a polypeptide having the amino acid sequence of residues Glu-223 to Tyr-246 of SEQ ID NO:2.

[0534] Additional embodiments of the invention are directed to the 9B6 antibody and to the hybridoma cell line expressing this antibody. A hybridoma cell line expressing Antibody 9B6 was deposited with the ATCC on January 7, 2000 and has been assigned ATCC Deposit No. PTA-1159. In a preferred embodiment, antibody 9B6 is humanized.

[0535] Additional embodiments of the invention are directed to the 15C10 antibody and to the hybridoma cell line expressing this antibody. A hybridoma cell line expressing Antibody 15C10 was deposited with the ATCC on January 7, 2000 and has been assigned ATCC Deposit No. PTA-1158. In a preferred embodiment, antibody 15C10 is humanized.

[0536] In a specific embodiment, the specific antibodies described above are humanized using techniques described herein or otherwise known in the art and then used as therapeutics as described herein.

[0537] In another specific embodiment, any of the antibodies listed above are used in a soluble form.

[0538] In another specific embodiment, any of the antibodies listed above are conjugated to a toxin or a label (as described infra). Such conjugated antibodies are used to kill a particular population of cells or to quantitate a particular population of cells. In a preferred embodiment, such conjugated antibodies are used to kill B cells expressing Neutrokine-alpha receptor on their surface. In another preferred embodiment, such conjugated antibodies are used to quantitate B cells expressing Neutrokine-alpha receptor on their surface. In another preferred embodiment, such conjugated antibodies are used to kill monocyte cells expressing the membrane-bound form of Neutrokine-alpha. In another preferred embodiment, such conjugated antibodies are used to quantitate monocyte cells expressing the membrane-bound form of Neutrokine-alpha and/or Neutrokine-alphaSV. In highly preferred embodiments, such conjugated antibodies that bind the membrane bound form of Neutokine-alpha and/or Neutrokine-alphaSV are used to kill Acute Mylogenous Leukemia cells, Chronic Lymphocytic leukemia cells, Multiple Myeloma cells, Non-Hodgkin's Lymphoma cells, and Hodgkins's lymphoma cells.

[0539] The antibodies of the invention also have uses as therapeutics and/or prophylaetics which include, but are not limited to, in activating monocytes or blocking monocyte activation and/or killing monocyte lineages that express the membrane bound form of Neutrokine-alpha on their cell surfaces (e.g., to treat, prevent, and/or diagnose myeloid leukemias, monocyte based leukemias and lymphomas, monocytosis, monocytopenia, rheumatoid arthritis, and other diseases or conditions associated with activated monocytes). In a specific embodiment, the antibodies of the invention fix complement. In other specific embodiments, as further described herein, the antibodies of the invention (or fragments thereof) are associated with heterologous polypeptides or nucleic acids (e.g. toxins, such as, compounds that bind and activate endogenous cytotoxie effecter systems, and radioisotopes; and cytotoxic prodrugs).

[0540] In another embodiment, one or more monoclonal antibodies are produced wherein they recognize or bind Neutrokine-alpha and/or a mutein thereof, but do not recognize or bind Neutrokine-alphaSV and/or a mutein thereof. In a related embodiment, one or more monoclonal antibodies are produced wherein they recognize or bind Neutrokine-alphaSV and/or a mutein thereof, but do not recognize or bind Neutrokine-alpha and/or a mutein thereof.

[0541] As discussed above, antibodies to the Neutrokine-alpha and/or Neutrokine-alpha SV polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the Neutrokine-alpha, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444 (1989), and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to Neutrokine-alpha and/or Neutrokine-alpha SV and competitively inhibit the Neutrokine-alpha and/or Neutrokine-alpha SV multimerization and/or binding to ligand can be used to generate anti-idiotypes that "mimie" the Neutrokine-alpha TNF mutimerization and/or binding domain and, as a consequence, bind to and neutralize Neutrokine-alpha or Neutrokine-alpha SV and/or its ligand. Sueh neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize Neutrokine-alpha ligand. For example, sueh anti-idiotypic antibodies can be used to bind Neutrokine-alpha and/or Neutrokine-alpha SV, or to bind Neutrokine-alpha and/or Neutrokine-alpha SV receptors on the surface of cells of B cell lineage, and thereby

block Neutrokine-alpha and/or Neutrokine-alpha SV mediated B cell activation, proliferation, and/or differentiation.

Immune System-Related Disorder Diagnosis

[0542] Neutrokine-alpha is expressed in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue, and particularly cells of monocytic lineage. Moreover, Neutrokine-alphaSV is expressed in primary dendritic cells. Additionally, Neutrokine-alpha is expressed on the cell surface of the following non-hematopoietic tumor cell lines. Colon carcinomas HCT 116 (ATCC Accession No. CCL-247) and HT-29 (ATCC Accession No. HTB-38); Colon adenocarcinomas Caco-2 (ATCC Accession No. HTB-37), COLO 201 (ATCC Accession No. CCL-224), and WiDr (ATCC Accession No. CCL-218); Breast adenocarcinoma MDA-MB-231 (ATCC Accession No. HTB-26); Bladder squamous carcinoma SCaBER (ATCC Accession No. HTB-3); Bladder carcinoma HT-1197 (ATCC Accession No. CRL-1473); Kidney carcinomas A-498 (ATCC Accession No. HTB-44), Caki-1 (ATCC Accession No. HTB-46), and Caki-2 (ATCC Accession No. HTG-47); Kidney, Wilms tumor SK-NEP-1 (ATCC Accession No. HTB-48); and Pancreas carcinomas Hs 766T (ATCC Accession No. HTB-134), MIA PaCa-2 (ATCC Accession No. CRL-1420), and SU.86.86 (ATCC Accession No. CRL-1837). For a number of immune system-related disorders, substantially altered (increased or decreased) levels of Neutrokine-alpha and/or Neutrokine-alphaSV gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Neutrokine-alpha and/or Neutrokine-alphaSV gene expression level, that is, the Neutrokine-alpha and/or Neutrokine-alphaSV expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, which involves measuring the expression level of the gene encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokine-alpha and/or Neutrokine-alphaSV gene expression level, whereby an increase or decrease in the gene expression level compared

to the standard is indieative of an immune system disorder or normal aetivation, proliferation, differentiation, and/or death.

[0543] In particular, it is believed that certain tissues in mammals with cancer of cells or tissue of the immune system express signifieantly enhanced or reduced levels of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide and mRNA encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide when compared to a corresponding "standard" level. Further, it is believed that enhanced or depressed levels of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) or eells or tissue from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

[0544] For example, as disclosed herein, Neutrokine-alpha is highly expressed in cells of monocytic lineage. Accordingly, polynucleotides of the invention (e.g., polynueleotide sequences complementary to all or a portion of Neutrokine-alpha mRNA and/or Neutrokine-alphaSV mRNA) and antibodies (and antibody fragments) directed against the polypeptides of the invention may be used to quantitate or qualitate concentrations of cells of monocytic lineage (e.g., monocytic leukemia cells) expressing Neutrokine-alpha on their cell surfaces. These antibodies additionally have diagnostic applications in detecting abnormalities in the level of Neutrokine-alpha gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of Neutrokine-alpha and/or Neutrokine-alphaSV. These diagnostic assays may be performed in vivo or in vitro, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[0545] Additionally, as disclosed herein, Neutrokine-alpha receptor is expressed primarily on cells of B cell lineage. Accordingly, Neutrokine-alpha polypeptides of the invention (ineluding labeled Neutrokine-alpha polypeptides and Neutrokine-alpha fusion proteins), and anti-Neutrokine-alpha antibodies (ineluding anti-Neutrokine-alpha antibody fragments) against the polypeptides of the invention may be used to quantitate or qualitate eoneentrations of cells of B cell lineage (e.g., B cell related leukemias or lymphomas) expressing Neutrokine-alpha receptor on their cell surfaces.

[0546] Neutrokine-alpha polypeptides and antibodies additionally have diagnostic applications in detecting abnormalities in the level of Neutrokine-alpha receptor gene expression (e.g., transmembrane activator and CAML interactor (TACI, GenBank

accesion number AAC51790), and B-cell maturation antigen (BCMA, GenBank accession number NP_001183)), or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of Neutrokine-alpha receptor and/or diagnosing activity/defects in signalling pathways associated with Neutrokine-alpha. These diagnostic assays may be performed in vivo or in vitro, such as, for example, on blood samples or biopsy tissue using techniques described herein or otherwise known in the art.

[0547] In one embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, prevent, diagnose, or prognose an individual having an immunodeficiency.

[0548] Immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed with the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention, include, but are not limited to one or more immunodeficiencies selected from: severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), chronic granulomatous disease, Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic alymphoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside

phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0549] According to this embodiment, an individual having an immunodeficiency expresses aberrantly low levels of Neutrokine-alpha and/or Neutrokine-alpha SV when compared to an individual not having an immunodeficiency. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample.

[0550] A biological sample of a person afflicted with an immunodeficiency is characterized by low levels of expression of Neutrokine-alpha and/or Neutrokine-alphaSV when compared to that observed in individuals not having an immunodeficiency. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an immunodeficiency. For example, a biological sample obtained from a person suspected of being afflicted with an immunodeficiency ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an immunodeficiency. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with an immunodeficiency.

[0551] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose and/or prognose an individual having common

variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease. According to this embodiment, an individual having CVID or a subset of individuals having CVID expresses aberrant levels of Neutrokine-alpha and/or Neutrokine-alpha Reeeptor on their B cells and/or monocytes, when compared to individuals not having CVID. Any means described herein or otherwise known in the art may be applied to deteet Neutrokine-alpha polynucleotides or polypeptides of the invention and/or Neutrokine-alpha Receptor polypeptides (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR deteetion of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine differentially the expression profile of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or Neutrokine-alpha receptor polypeptides in a sample containing at least monocyte cells or some component thereof (e.g., RNA) as compared to a sample containing at least B cells or a component thereof (e.g., RNA). In the instance where a sample containing at least monocyte cells or some component thereof (e.g., RNA) is determined to reflect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotide or polypeptide expression and a sample containing at least B cells or a component thereof (e.g., RNA) is determined to reflect less than normal levels of Neutrokine-alpha receptor polynucleotide or polypeptide expression, the samples may be correlated with the occurrence of CVID (i.e., "acquired agammaglobulinemia" or "acquired hypogammaglobulinemia").

[0552] A subset of persons afflicted with CVID are characterized by high levels of expression of both Neutrokine-alpha and the Neutrokine-alpha receptor ("NAR") in peripheral or circulating B cells when compared to that observed in individuals not having CVID. In contrast, persons who are not afflieted with CVID are typically characterized by low levels of Neutrokine-alpha expression and high levels of NAR expression in peripheral or circulating B cells. Thus, Neutrokine-alpha, Neutrokine-alphaSV polypeptides, and/or NAR polypeptides, polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the differential diagnosis of this subset of CVID. For example, a sample of peripherial B cells obtained from a person suspected of being afflicted with CVID ("the

subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, Neutrokine-alphaSV, and/or NAR polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflieted with CVID ("the control"). A significant difference in expression level(s) of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, and/or NAR polypeptides, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with this subset of CVID.

[0553] Cunningham-Rundles and Bodian followed 248 CVID patients over a period of 1-25 years and discovered that a number of associated diseases or conditions appear with increased frequency in CVID patients (Cunningham-Rundles and Bodian, *J. Clin. Immunol.*, 92:34-48 (1999) which is herein incorporated by reference in its entirety.) The most important clinical events include infections, autoimmunity, inflammatory disorders, marked by gastrointestinal and granulomatous disease, cancer and hepatitis. Most CVID patients are at increased risk of recurrent infections particularly of the respiratory tract. The types of acute and recurring bacterial infections exhibited in most patients include pneumonia, bronchitis and sinusitis. Children with CVID have a marked increased risk of otitis media. Additionally, blood borne infections including sepsis, meningitis, septic arthritis, and osteomyelitis are seen with increased frequency in these patients.

[0554] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSVantibodies) are used to diagnose, prognose, treat, or prevent conditions associated with CVID, including, but not limited to, conditions associated with acute and recurring infections (e.g., pneumonia, bronchitis, sinusitis, otitis media, sepsis, meningitis, septic arthritis, and osteomyelitis), chronic lung disease, autoimmunity, granulomatous disease, lymphoma, cancers (e.g., cancers of the breast, stomach, colon, mouth, prostate, lung, vagina, ovary, skin, and melanin forming eells (i.e. melanoma), inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, and ulcerative proctitis), malabsoption, Hodgkin's disease, and Waldenstrom's maeroglobulinemia.

[0555] In a specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSVantibodies) are used to diagnose, prognose, treat, or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) may be used to diagnose, prognose, treat, or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carnii.

[0556] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having an autoimmune disease or disorder.

[0557] Autoimmune diseases or disorders that may be treated, diagnosed, or prognosed using Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention include, but are not limited to, one or more of the following: autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g, IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye,

autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erhythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease , (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura , rheumatoid arthritis, schleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernieious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes millitus, and adrenergie drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiotomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulamatous, degenerative, and atrophic disorders.

[0558] According to this embodiment, an individual having an autoimmune disease or disorder expresses aberrantly high levels of Neutrokine-alpha, Neutrokine-alpha SV, and/or NAR when compared to an individual not having an autoimmune disease or disorder. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or NAR polypeptides (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention and/or NAR polypeptides in a biological sample.

[0559] A biological sample of persons afflieted with an autoimmune disease or disorder is charaeterized by high levels of expression of Neutrokine-alpha, Neutrokine-alphaSV, and/or NAR when compared to that observed in individuals not having an autoimmune disease or disorder. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynueleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an autoimmune disease or disorder. For example, a biological sample obtained from a person suspected of being afflieted with an autoimmune disease or disorder ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-

alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention and/or NAR polypeptides. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an autoimmune disease or disorder. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, and/or NAR polypeptides between samples obtained from the subject and the control suggests that the subject is afflicted with an autoimmune disease or disorder.

[0560] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having systemic lupus erythematosus or a subset of this disease. According to this embodiment, an individual having systemic lupus erythematosus or a subset of individuals having systemic lupus erythematosus expresses aberrantly high levels of Neutrokine-alpha and/or Neutrokine-alpha SV when compared to an individual not having systemic lupus erythematosus or this subset of systemic lupus erythematosus. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample .

[0561] A biological sample of persons afflicted with systemic lupus erythematosus is characterized by high levels of expression of Neutrokine-alpha and/or Neutrokine-alphaSV when compared to that observed in individuals not having systemic lupus erythematosus. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of systemic lupus erythematosus or a subset of systemic lupus erythematosus. For example, a

biological sample obtained from a person suspected of being afflicted with systemic lupus erytheamatosus ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with systemic lupus erythematosus. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with systemic lupus erythematosus or a subset thereof.

[0562] Furthermore, there is a direct correlation between the severity of systemic lupus erythematosus, or a subset of this disease, and the concentration of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides (RNA) and/or polypeptides of the invention. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides, (RNA), polypeptides and/or agonists or antagonists of the invention, may be used according to the methods of the invention in prognosis of the severity of systemic lupus erythematosus or a subset of systemic lupus erythematosus. For example, a biological sample obtained from a person suspected of being afflicted with systemic lupus erythematosus ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a panel of persons known to represent a range in severities of this disease. According to this method, the match of expression level with a characterized member of the panel indicates the severity of the disease.

[0563] Elevated levels of soluble Neutrokine-alpha have been observed in the serum of patients with Systemic Lupus Erythematosus (SLE). In comparing the sera of 150 SLE patients with that of 38 control individuals, it was found that most of the SLE patients had more than 5ng/ml of serum Neutrokine-alpha, more than 30% of SLE patients had levels greater than 10ng/ml, and approximately 10% of SLE patients had serum Neutrokine-alpha levels greater than 20ng/ml. In contrast, the majority of normal controls had Neutrokine-alpha levels less than 5ng/ml, and less than 10% had levels higher than

10ng/ml. The elevated levels of Neutrokine-alpha protein in sera is present in the soluble form and has biologic activity as assayed by the ability to stimulate anti-IgM treated B cells in vitro. SLE patients with more than 15ng/ml serum Neutrokine-alpha were also found to have elevated levels of anti-dsDNA antibodies compared to both normal controls and SLE patients with less than 5ng/ml of serum Neutrokine-alpha (unpublished data).

[0564] In addition the serum of two subgroups of patients whieh were positive for anti-nuclear antibodies (ANA+) but did not meet the formal requirements of the American College of Rheumatology (ACR) for classifieation of SLE were anaylzed for Neutrokine-alpha levels. The first subgroup of sera was ANA+ sera that came from patients who did not present with the elinical impression of SLE. This group had only slightly elevated levels of Neutrokine-alpha (~9ng/ml Neutrokine-alpha). The second subgroup however, which was ANA+ sera from patients who presented with the clinical impression of SLE, had significantly increased Neutrokine-alpha levels (~15ng/ml). These results suggest that an elevated level of Neutrokine-alpha precedes the formal fulfillment of the ACR criteria. The ACR criteria are desrcibed in Tan, E.M., et al, *Arthritis and Rheumatism* 25:1271 – 1277 (1982).

[0565] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having rheumatoid arthritis or a subset of this disease. According to this embodiment, an individual having rheumatoid arthritis or a subset of individuals having rheumatoid arthritis expresses aberrantly high levels of Neutrokine-alpha and/or Neutrokine-alpha SV when compared to an individual not having rheumatoid arthritis or this subset of rheumatoid arthritis. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample .

[0566] A biological sample of persons afflicted with rheumatoid arthritis is characterized by high levels of expression of Neutrokine-alpha and/or Neutrokine-alphaSV when compared to that observed in individuals not having rheumatoid arthritis. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of rheumatoid arthritis or a subset of rheumatoid arthritis. For example, a biological sample obtained from a person suspected of being afflicted with rheumatoid arthritis ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with rheumatoid arthritis. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with rheumatoid arthritis or a subset thereof.

[0567] In other specific embodiments, antibodies of the invention which specifically bind to Neutrokine-alpha and/or Neutrokine-alphaSV can be used for diagnostic purposes to detect, diagnose, prognose, or monitor Sjögren's Syndrome or conditions associated therewith. The invention provides for the detection of aberrant expression of Neutrokine-alpha and/or Neutrokine-alphaSV comprising: (a) assaying the expression of Neutrokine-alpha and/or Neutrokine-alphaSV in a biological sample of an individual using one or more antibodies of the invention that immunospecifically binds to Neutrokine-alpha and/or Neutrokine-alphaSV; and (b) comparing the level of Neutrokine-alpha and/or Neutrokine-alphaSV with a standard level of Neutrokine-alpha and/or Neutrokine-alphaSV, *e.g.*, in normal biological samples, whereby an increase in the assayed level of Neutrokine-alpha and/or Neutrokine-alphaSV compared to the standard level of Neutrokine-alpha and/or Neutrokine-alphaSV is indicative of Sjögren's Syndrome.

[0568] In other specific embodiments, antibodies of the invention which specifically bind to Neutrokine-alpha and/or Neutrokine-alphaSV can be used for diagnostic purposes to detect, diagnose, prognose, or monitor HIV infection or conditions associated therewith

(e.g., AIDS) The invention provides for the detection of aberrant expression of Neutrokine-alpha and/or Neutrokine-alphaSV comprising: (a) assaying the expression of Neutrokine-alpha and/or Neutrokine-alphaSV in a biological sample of an individual using one or more antibodies of the invention that immunospecifically binds to Neutrokine-alpha and/or Neutrokine-alphaSV; and (b) comparing the level of Neutrokine-alpha and/or Neutrokine-alphaSV with a standard level of Neutrokine-alpha and/or Neutrokine-alphaSV, *e.g.*, in normal biological samples, whereby an increase in the assayed level of Neutrokine-alpha and/or Neutrokine-alphaSV compared to the standard level of Neutrokine-alpha and/or Neutrokine-alphaSV is indicative of HIV infection.

[0569] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual with an immune-based rheumatologic diseases, including but not limited to, SLE, rheumatoid arthritis, CREST syndrome (a variant of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.), seronegative spondyloarthropathy (SpA), polymyositis/dermatomyositis, microscopic polyangiitis, hepatitis C-asociated arthritis, Takayasu's arteritis, and undifferentiated connective tissue disorder. According to this embodiment, an individual having an immune-based rheumatologic disease or a subset of individuals having a particular immune-based rheumatologic disease expresses aberrantly high levels of Neutrokine-alpha and/or Neutrokine-alpha SV when compared to an individual not having the particular immune-based rheumatologic disease or this subset of individuals having the particular immune-based rheumatologic disease. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample .

[0570] A biological sample of persons afflicted with an immune-based rheumatologic disease is characterized by high levels of expression of Neutrokine-alpha and/or

Neutrokine-alphaSV when compared to that observed in individuals not having an immune-based rheumatologic disease. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an immune-based rheumatologic disease. For example, a biological sample obtained from a person suspected of being afflicted with an immune-based rheumatologic disease ("the subject") may be analyzed for the relative expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an immune-based rheumatologic disease. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with an immune-based rheumatologic disease.

[0571] It has been observed, that serum Neutrokina-alpha levels inversely correlate with nephrotic-range proteinuria (>3gm proteinuria in a 24 hour urine collection) using a sample of 71 SLE patients (p=0.019). Proteinuria was determined in 71 SLE patients within one month of phlebotomy for serum Neutrokine-alpha determination. Serum Neutrokine-alpha was classified as low, normal, or high based on the 5^{th} through 95^{th} percentiles for normal controls. Nephrotic-range proteinuria was inversely correlated with serum Neutrokine-alpha levels. Thus, in specific embodiments, serum levels of Neutrokine-alpha in individuals diagnosed with an immune based rheumatologic disease (e.g., SLE, rheumatoid arthritis, CREST syndrome (a variant of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.), seronegative spondyloarthropathy (SpA), polymyositis/dermatomyositis, microscopic polyangiitis, hepatitis C-asociated arthritis, Takayasu's arteritis, and undifferentiated connective tissue disorder) may be used to determine, diagnose, progonose, or monitor the severity of certain aspects or symptoms of the disease, such as nephrotic-range proteinuria.

[0572] Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, including cancers of this system, and immunodeficiencies and/or autoimmune diseases which involves measuring the expression level of the gene encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokine-alpha and/or Neutrokine-alphaSV gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

[0573] Levels of soluble Neutrokine-alpha in the serum of patients with follicular non-Hodgkin's lymphoma are elevated elevated compared to levels of soluble neutrokine-alpha in the sera of healthy individuals. Thus, in a specific embodiment, the invention provides method of diagnosing non-Hodgkin's lymphoma which involves measuring the expression level of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides and/or polynucleotides in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokine-alpha and/or Neutrokine-alphaSV gene expression level, whereby an increase in the gene expression level compared to the standard is indicative of non-Hodgkin's Lymphoma. Other forms of Non-Hodgkin's lymphoma which may be diagnosed according to the above method include, but are not limited to, mantle cell lymphoma, diffuse large cell lymphoma, chronic lymphocytic leukemia, small lymphocytic leukemia, and marginal zone lymphoma.

[0574] Where a diagnosis of a disorder in the immune system, including, but not limited to, diagnosis of a tumor, diagnosis of an immunodeficiency, and/or diagnosis of an autoimmune disease, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed Neutrokine-alpha and/or Neutrokine-alphaSV gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0575] By analyzing or determining the expression level of the gene encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is intended qualitatively or quantitatively measuring or estimating the level of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide or the level of the mRNA encoding the Neutrokine-alpha

and/or Neutrokine-alphaSV polypeptide in a first biological sample either direetly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA levcl in a second biological sample). Preferably, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level, the standard being taken from a seeond biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0576] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which eontains Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the Neutrokine-alpha and/or Neutrokine-alphaSV or a Neutrokine-alpha and/or Neutrokine-alphaSV receptor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred souree.

[0577] The eompounds of the present invention are useful for diagnosis, prognosis, or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include, but are not limited to tumors (e.g., B cell and monocytic cell leukemias and lymphomas, See Example) and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases (e.g., rheumatoid arhtritis, systemie lupus erythamatosus, Sjogren syndrome, mixed connective tissue disease, and inflammatory myopathies), and graft versus host disease.

[0578] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0579] Assaying Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide levels in a biological sample can occur using antibody-based techniques. For example, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)). Other antibody-based methods useful for detecting Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluoresence activated cell sorting (FACS). Suitable antibody assay labels are known in the art and include enzyme labels, (e.g., glucose oxidase, alkaline phosphatase and horse radish peroxidase) and radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0580] Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter, Chloramine-T reaction, and Iodogen®-based labelling).

[0581] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the Neutrokine-alpha gene (such as, for example, cells of monocytic lineage) or cells or tissue which are known, or suspected, to express the Neutrokine-alpha reeeptor gene (such as, for example, cells of B cell lineage and the spleen). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), whieh is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a eell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the Neutrokine-alpha gene or Neutrokine-alpha receptor gene.

[0582] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of Neutrokine-alpha gene products or eonserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody eoupled with light microscopic, flow cytometric, or fluorimetrie detection.

[0583] The antibodies (or fragments thereof) or Neutrokine-alpha polypeptides or polypeptides of the present invention may, additionally, be employed histologically, as in immunofluorescenee, immunoeleetron microscopy or non-immunological assays, for in situ detection of Neutrokine-alpha gene produets or conserved variants or peptide fragments thereof, or for Neutrokine-alpha binding to Neutrokine-alpha receptor. In situ detection may be accomplished by removing a histologieal specimen from a patient, and applying thereto a labeled antibody or Neutrokine-alpha polypeptide of the present invention. The antibody (or fragment) or Neutrokine-alpha polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the Neutrokine-alpha gene product, or conserved variants or peptide fragments, or Neutrokine-alpha polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide

variety of histologieal methods (such as staining procedures) can be modified in order to achieve sueh in situ detection.

[0584] Immunoassays and non-immunoassays for Neutrokine-alpha gene products or conserved variants or peptide fragments thereof will typically comprise ineubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of eells which have been incubated in eell culture, in the presence of a detectably labeled antibody eapable of identifying Neutrokine-alpha gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0585] Immunoassays and non-immunoassays for Neutrokine-alpha receptor gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, sueh as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectable or labeled Neutrokine-alpha polypeptide capable of identifying Neutrokine-alpha reeeptor gene produets or eonserved variants or peptide fragments thereof, and detecting the bound Neutrokine-alpha polypeptide by any of a number of techniques well-known in the art.

[0586] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell partieles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled anti-Neutrokine-alpha antibody or detectable Neutrokine-alpha polypeptide. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0587] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible struetural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or eylindrical, as in the inside surface of a test tube, or the external

surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0588] The binding activity of a given lot of anti-Neutrokine-alpha antibody or Neutrokine-alpha polypeptide may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0589] In addition to assaying Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides can also be detected in vivo by imaging. For example, in one embodiment of the invention, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide and/or anti-Neutrokine-alpha antibody is used to image B cell lymphomas. In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypepitdes and/or anti-Neutrokine-alpha antibodies and/or Neutrokine-alpha polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of Neutrokine-alpha and/or Neutrokine-alphaSV mRNA) is used to image lymphomas (e.g., monoeyte and B cell lymphomas) .

[0590] Antibody labels or markers for *in vivo* imaging of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inelude those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or eesium, which emit detectable radiation but are not overtly harmful to the subjeet. Suitable markers for NMR and ESR inelude those with a detectable characteristic spin, such as deuterium, whieh may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoelonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et

al., WO 8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al., Nature 314*:268 (1985).

[0591] Additionally, any Neutrokine-alpha polypeptide whose presence can be detected, can be administered. For example, Neutrokine-alpha polypeptides labeled with a radio-opaque or other appropriate compound can be administered and visualized in vivo, as discussed, above for labeled antibodies. Further such Neutrokine-alpha polypeptides can be utilized for in vitro diagnostic procedures.

[0592] A Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide-specific antibody or antibody fragment whieh has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain Neutrokine-alpha protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0593] With respect to antibodies, one of the ways in which the anti-Neutrokine-alpha antibody can be detectably labeled is by linking the same to an enzyme and using the linked produet in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. et al., (eds.),

1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0594] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect Neutrokine-alpha through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0595] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave-length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0596] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0597] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is

then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0598] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presenee of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling include, but are not limited to, lueiferin, luciferase and aequorin.

Treatment of Immune System-Related Disorders

[0599] As noted above, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and polypeptides, and anti-Neutrokine-alpha antibodies, are useful for diagnosis of conditions involving abnormally high or low expression of Neutrokine-alpha and/or Neutrokine-alphaSV activities. Given the cells and tissues where Neutrokine-alpha and/or Neutrokine-alphaSV is expressed as well as the activities modulated by Neutrokine-alpha and/or Neutrokine-alphaSV, it is readily apparent that a substantially altered (inereased or decreased) level of expression of Neutrokine-alpha and/or Neutrokine-alphaSV in an individual compared to the standard or "normal" level produces pathological conditions related to the bodily system(s) in which Neutrokine-alpha and/or Neutrokine-alphaSV is expressed and/or is active.

[0600] It will also be appreciated by one of ordinary skill that, since the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention are members of the TNF family, the extraeellular domains of the respective proteins may be released in soluble form from the cells which express Neutrokine-alpha and/or Neutrokine-alphaSV by proteolytie cleavage and therefore, when Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide (particularly a soluble form of the respective extraeellular domains) is added from an exogenous souree to cells, tissues or the body of an individual, the polypeptide will exert its modulating activities on any of its target cells of that individual. Also, cells expressing this type II transmembranc protein may be added to cells, tissues or the body of an individual whereby the added cells will bind to cells expressing receptor for

Neutrokine-alpha and/or Neutrokine-alphaSV whereby the cells expressing Neutrokine-alpha and/or Neutrokine-alphaSV can cause responses (e.g., proliferation or cytotoxicity) in the receptor-bearing target cells.

[0601] In one embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies assoeiated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells, such as, for example, B eells expressing Neutrokine-alpha and/or Neutrokine-alphaSV receptor, or monocytes expressing the cell surface bound form of Neutrokine-alpha and/or Neutrokine-alphaSV. Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleie aeids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or eovalent interactions.

[0602] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to eells by administering polypeptides of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) that are associated with heterologous polypeptides or nucleic aeids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0603] In another embodiment, the invention provides for a method of killing eells of hematopoietic origin, comprising, or alternatively consisting of, contacting Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides with cells of hematopoietic origin. In speeific embodiments, the method of killing cells of hematopoietic origin, comprises, or alternatively consists of, administering to an animal in which such killing is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effeetive to kill cells of hematopoietic origin. Cells of hematopoietic origin include, but are not limited to, lymphocytes (e.g., B cells and T cells), monocytes, macrophages, dendritic cells, polymorphonuelear leukocytes (e.g., basophils, eosinophils, neutrophils), mast cells,

platelets, erythrocytes and progenitor cells of these lineages. Cells of hematopoietic origin include, but are not limited to, healthy and diseased cell as found present in an animal, preferably a mammal and most preferably a human, or as isolated from an animal, transformed cells, cell lines derived from the above listed cell types, and cell cultures derived from the above listed cell types. Cells of hematopoietic origin may be found or isolated in, for example, resting, activated or anergic states.

[0604] In another embodiment, the invention provides a method for the specific destruction (i.e., killing) of cells (e.g., the destruction of tumor cells) by administering Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide conjugates of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides conjugated with radioisotopes, toxins, or cytotoxic prodrugs) in which such destruction of cells is desired.

[0605] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) in association with toxins or cytotoxic prodrugs.

[0606] In a specific embodiment, the invention provides a method for the specific destruction of cells of B cell lineage (e.g., B cell related leukemias or lymphomas) by administering Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides in association with toxins or cytotoxic prodrugs.

[0607] In another specific embodiment, the invention provides a method for the specific destruction of cells of monocytic lineage (e.g., monocytic leukemias or lymphomas) by administering anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies in association with toxins or cytotoxic prodrugs.

[0608] Biodistribution studies (See Example 12) of radiolabeled Neutrokine-alpha polypeptide (amino acids 134-285 of SEQ ID NO:2) that had been injected into BALB/c mice demonstrated that Neutrokine-alpha has high in vivo targeting specificity for lymphoid tissues such as spleen and lymph nodes. Thus in specific embodiments, the invention provides a method for the specific destruction or disablement of lymphoid tissue (e.g., lymph nodes and spleen) by administering Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with

radioisotopes, toxins or cytotoxic prodrugs. In preferred embodiments, the lymphoid tissue is not permantly destroyed, but rather is temporarily disabled, (e.g, cells of hematopoietic lineage in lymphoid tissues are destroyed/killed while Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are administered, but these populations recover once administration of Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs is stopped.)

[0609] By "toxin" is meant compounds that bind and aetivate endogenous cytotoxic effeetor systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, eytotoxins (cytotoxic agents), or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the eell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, eompounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a eytostatic or cytoeidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium; luminescent labels, such as luminol; and fluorcseent labels, such as fluorescein and rhodamine, and biotin.

[0610] Tcchniqucs known in the art may be applied to label polypeptides and antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter, Chloramine-T reaction, and Iodogen® based labeling methods).

[0611] A cytotoxin or cytotoxic agent includes any agent that is detrimental to eells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine,

mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mereaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiocpa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotie agents (e.g., vincristine and vinblastine).

[0612] By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

[0613] In specific embodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of autoimmune diseases. In preferred emodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in assoeiation with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of systemic lupus erythematosus. Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of rheumatoid arthritis including advanced rheumatoid arthritis. In preferred emodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of idiopathic thrombocytopenic purpura (ITP).

[0614] In other preferred embodiments Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of Sjögren's syndrome. In other preferred embodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of IgA nephropathy. In other preferred embodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of Myasthenia gravis. In preferred emodiments, Neutrokine-alpha , Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of multiple sclerosis. In still other preferred embodiments, Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of vasculitis.

[0615] In one embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduces Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production. In specific embodiments, the invention provides methods and compositions for inhibiting or redueing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens, comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduces Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production in response to T cell dependent antigens. In specific embodiments, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or

IgA produetion) in response to T cell independent antigens, comprising, or alternatively eonsisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduces Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production in response to T cell independent antigens.

[0616] In another embodiment, the invention provides methods and compositions for inhibiting or redueing immunoglobulin production (e.g. IgM, IgG, and/or IgA production), eomprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to inhibit or reduce immunoglobulin production. In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens, comprising, or alternatively eonsisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to inhibit or reduce immunoglobulin production in response to T cell dependent antigens. In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effeetive to inhibit or reduce immunoglobulin production in response to T cell independent antigens.

[0617] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contaeting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide stimulates Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens comprising, or alternatively consisting of,

contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide stimulates Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production in response to T cell dependent antigens. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin produetion (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens comprising, or alternatively consisting of, contacting an effeetive amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide stimulates Neutrokine-alpha and/or Neutrokine-alphaSV mediated immunoglobulin production in response to T cell independent antigens.

[0618] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effeetive to stimulate immunoglobulin production. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to stimulate immunoglobulin production in response to T cell dependent antigens.

[0619] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin produetion (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to stimulate immunoglobulin production in response to T cell independent antigens.

[0620] Determination of immunoglobulin levels are most often performed by comparing the level of immunoglobulin in a sample to a standard containing a known amount of immunoglobulin using ELISA assays. Determination of immunoglobulin

levels in a given sample, can readily be determined using ELISA or other method known in the art.

[0621] Receptors belonging to the TNF receptor (TNFR) super family (e.g., TACI and BCMA, receptors to which Neutrokine-alpha polypeptides bind) can be classified into two types based on the presenee or absence of a conserved cytoplasmic domain responsible for apoptosis ealled a "death domain." TNF receptors without death domains, such as TNF-R2 HVEM/ATAR, RANK, CD27, CD30, CD40, and OX40 interact with TNF receptor associated factors (TRAF 1-6) and mediate anti-apoptotic survival and or proliferative responses via activation of the transcription factor NF-kappaB (reviewed in Wajant et al., Cytokine and Growth Factor Reviews 10(1):15-26, 1999). TACI and BCMA do not contain death domains.

[0622] Investigation of Neutrokine-alpha (which bind TACI and BCMA) indueed signaling in human tonsillar B cells co-stimulated with Staph. Aureus Cowan consistently revealed that mRNA for ERK-1 and PLK were upregulated by Neutrokine-alpha + SAC treatment (see Example 11). Polo like kinases (PLK) belong to a sub family of serine/threonine kinases related to Saceharomyces cerevisiae eell cycle protein CDC5 (29). The expression of PLK is induced during G2 and S phase of the cell eyele. PLK is reported to play a role in cell proliferation (Lee et al., Proe. Natl. Acad. Sei. 95:9301 – 9306). The role or extracellular-signal related kinases (ERK1/2) in eell survival and proliferative effects of growth faetors and other agonists has been extensively studied. The induced expression of PLK and ERK-1 is consistent with the survival and proliferative effects of Neutrokine-alpha on B cells.

[0623] Additionally, in some samples of human tonsillar B cells stimulated with Neutrokine-alpha and SAC, mRNA for CD25 (IL-2Ralpha) was upregulated. Nuclear extracts from Human tonsillar B cells treated with Neutrokine-alpha and from IM-9 cells treated with Neutrokine-alpha were able to shift probes from the CD25 promoter region containing sites for NF-kappaB, SRF, ELF-1 and HMGI/Y in an electromobility shift assay. ELF-1 for example, is a transcription factor that is part of the ETS family of proteins and whose expression appears to be restricted to T and B cells. Binding sites for ELF-1 have been deseribed in the promoters of a number of proteins that are important in the regulation of the immune response.

[0624] Thus, Neutrokine-alpha induced signaling has been shown to be consistent with the activation of cellular activation and cellular proliferation pathways as well as with cellular signaling pathways that regulate B cell lifespan. Further, Neutrokine-alpha and/or Neutrokine-alphaSV treatment of B cells induces cellular proliferation immunoglobulin secretion, a characteristic of activated B cells (Moore et al., Science 285:260-263, 1999). Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides may inhibit, stimulate, or not significantly alter these Neutrokine-alpha and/or Neutrokine-alphaSV mediated activities.

[0625] In one embodiment, the invention provides methods and compositions for inhibiting or reducing proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduces Neutrokine-alpha and/or Neutrokine-alphaSV mediated proliferation of cells of hemtopoietic origin. In another embodiment, the invention provides methods and compositions for inhibiting or reducing reducing proliferation of cells of hematopoietic origin comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to inhibit or reduce B cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0626] In one embodiment, the invention provides methods and compositions for stimulating proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide stimulates Neutrokine-alpha and/or Neutrokine-alphaSV mediated proliferation of cells of hemtopoietic origin. In another embodiment, the invention provides methods and compositions for stimulating proliferation of cells of hematopoietic origin comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to stimulate B cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells. B cell proliferation is most commonly assayed in the art by measuring tritiated thymidine incorporation (see Examples 6 & 7). This and other assays are commonly known in the

art and could be routinely adapted for the use of determining the effect of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides on B cell proliferation.

[0627] In one embodiment, the invention provides methods and eompositions for inhibiting or reducing activation of cells of hematopoietic origin, comprising, or alternatively eonsisting of, eontacting an effeetive amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduees Neutrokine-alpha and/or Neutrokine-alphaSV mediated activation of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for inhibiting or redueing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which sueh inhibition or reduetion is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to inhibit or reduce aetivation of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0628] In one embodiment, the invention provides methods and compositions for inereasing aetivation of cells of hematopoietic origin, comprising, or alternatively eonsisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide increases Neutrokine-alpha and/or Neutrokine-alphaSV mediated activation of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for increasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to increase activation of eells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0629] B cell activation can measured in a variety of ways, such as FACS analysis of aetivation markers expressed on B cells. B cells activation markers include, but are not limited to, CD26, CD 28, CD 30, CD 38, CD 39, CD 69, CD70 CD71 , CD 77, CD 83, CD126, CDw130, and B220. Additionally, B cell activation may be measured by analysis of the activation of signaling molecules involved in B cell activation. By way of non-limiting example, such analysis may take the form of analyzing mRNA levels of signaling

molecules by Northern analysis or real time PCR (See Example 11). One can also measure, for example, the phosphorylation of signaling molecules using anti-phosphotyrosine antibodies in a Western blot. B cell activation may also be measured by measuring the calcium levels in B cells. These and other methods of determining B cell activation are commonly known in the art and could be routinely adapted for the use of determining the effect of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides on B cell activation.

[0630] In one embodiment, the invention provides methods and compositions for decreasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide inhibits or reduces Neutrokine-alpha and/or Neutrokine-alphaSV regulated lifespan of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for decreasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such decrease is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to decrease lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0631] In one embodiment, the invention provides methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide increases Neutrokine-alpha and/or Neutrokine-alphaSV regulated lifespan of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to increase lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0632] B cell life span in vivo may be measured by 5-bromo-2'-deoxyuridine (BrdU) labeling experiments which are well known to one skilled in the art. BrdU is a thymidine

analogue that gets incorporated into the DNA of dividing cells. Cells containing BrdU in their DNA can be detected using, for example fluorescently labeled anti-BrdU antibody and flow cytometry. Briefly, an animal is injected with BrdU in an amount suffieient to label developing B cells. Then, a sample of B cells is withdrawn from the animal, for example, from peripheral blood, and analyzed for the pereentage of cells that contain BrdU. Such an analysis performed at several time points can be used to calculate the half life of B cells. Alternatively, B cell survival may be measured in vitro. For example B cells may be cultured under conditions where proliferation does not occur, (for example the media should contain no reagents that crosslink the immunoglobulin receptor, such as anti-IgM antibodies) for a period of time (usually 2-4 days). At the end of this time, the pereent of surviving cells is determined, using for instance, the vital dye Trypan Blue, or by staining cells with propidium iodide or any other agent designed to specifically stain apoptotic cells and analyzing the percentage of cells stained using flow cytometry. One could perform this experiment under several conditions, such as B cells treated with Neutrokine-alpha, B cells treated with Neutrokine-alpha and/or Neutrokine-alphaSV-polypeptide complexes, and untreated B eells in order to determine the effects of Neutrokine-alpha and/or Neutrokine-alphaSV and Neutrokine-alpha polypeptides on B eells survival. These and other methods for determining B cell lifespan are commonly known in the art and could routinely be adapted to determining the effect of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides on Neutrokine-alpha and/or Neutrokine-alphaSV regulated B cell lifespan.

[0633] It will be appreciated that conditions caused by a decrease in the standard or normal level of Neutrokine-alpha and/or Neutrokine-alphaSV activity in an individual, particularly disorders of the immune system, ean be treated by administration of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide (in the form of soluble extracellular domain or cells expressing the complete protein) or agonist. Thus, the invention also provides a method of treatment of an individual in need of an inereased level of Neutrokine-alpha and/or Neutrokine-alphaSV activity comprising administering to such an individual a pharmaeeutical composition comprising an amount of an isolated Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide of the invention, or agonist thereof, effective to increase the Neutrokine-alpha and/or Neutrokine-alphaSV activity level in such an individual.

[0634] It will also be appreciated that conditions caused by a increase in the standard or normal level of Neutrokine-alpha and/or Neutrokine-alphaSV activity in an individual, particularly disorders of the immune system, can be treated by administration of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides (in the form of soluble extracellular domain or cells expressing the complete protein) or antagonist (e.g, an anti-Neutrokine-alpha antibody). Thus, the invention also provides a method of treatment of an individual in need of an decreased level of Neutrokine-alpha and/or Neutrokine-alphaSV activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide of the invention, or antagonist thereof, effective to decrease the Neutrokine-alpha and/or Neutrokine-alphaSV activity level in such an individual. A non-limiting example of a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide of the invention that can be administered to an individual in need of an decreased level of Neutrokine-alpha and/or Neutrokine-alphaSV activity, is a dominant negative mutant of a Neutrokine-alpha and/or Neutrokine-alphaSV, which binds to a Neutrokine-alpha and/or Neutrokine-alphaSV receptor but that does not induce signal transduction.

[0635] Autoantibody produetion is common to several autoimmune diseases and contributes to tissue destruction and exacerbation of disease. Autoantibodies can also lead to the oceurrence of immune complex deposition complications and lead to many symptoms of systemie lupus erythomatosis, including kidney failure, neuralgic symptoms and death. Modulating antibody production independent of eellular response would also be beneficial in many disease states. B cells have also been shown to play a role in the secretion of arthritogenic immunoglobulins in rheumatoid arthritis, (Korganow et al., Immunity 10:451-61, 1999) . As such, inhibition of Neutrokine alpha-mediated antibody production would be beneficial in treatment of autoimmune diseases such as myasthenia gravis and rheumatoid arthritis. Compounds of the invetion that selectively block or neutralize the action of B-lymphocytes would be useful for such purposes. To verify these capabilities in eompositions of the present invention, such compositions are evaluated using assays known in the art and described herein.

[0636] The invention provides methods employing compositions of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the

invention and/or agonists and/or antagonists thereof) for selectively blocking or neutralizing the actions of B-cells in association with end stage renal diseases, which may or may not be associated with autoimmune diseases. Such methods would also be useful for treating immunologic renal diseases. Such methods would be would be useful for treating glomerulonephritis associated with diseases such as membranous nephropathy, IgA nephropathy or Berger's Disease, IgM nephropathy, Goodpasture's Disease, post-infectious glomerulonephritis, mesangioproliferative disease, minimal-change nephrotic syndrome. Such methods would also serve as therapeutic applications for treating secondary glomerulonephritis or vasculitis associated with such diseases as lupus, polyarteritis, Henoch-Schonlein, Scleroderma, HIV-related diseases, amyloidosis or hemolytic uremic syndrome. The methods of the present invention would also be useful as part of a therapeutic application for treating interstitial nephritis or pyelonephritis assoeiated with chronic pyelonephritis, analgesic abuse, nephrocalcinosis, nephropathy eaused by other agents, nephrolithiasis, or chronic or acute interstitial nephritis.

[0637] The methods of the present invention also include use of compositions of the invention in the treatment of hypertensive or large vessel diseases, including renal artery stenosis or occlusion and cholesterol emboli or renal emboli.

[0638] The present invention also provides methods for diagnosis and treatment of renal or urological neoplasms, multiple mylelomas, lymphomas, light chain neuropathy or amyloidosis.

[0639] The invention also provides methods for blocking or inhibiting activated B cells using compositions of the invention for the treatment of asthma and other chronic airway diseases such as bronchitis and emphysema.

[0640] Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, or agonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, can be used in the treatment of infectious agents. For example, by increasing the immune response, particularly inereasing the proliferation and differentiation of B cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, may also directly inhibit the infectious agent, without necessarily elieiting an immune response.

[0641] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaceinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syneytial virus, encephalitis, eye infeetions (e.g., conjunctivitis, keratitis), chronie fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B eneephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagie fever, Measles, Mumps, Parainfluenza, Rabies, the common eold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat patients nonresponsive to one or more other commereially available hepatitis vaccines. In a further specific embodiment, Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose AIDS. In an additional specific embodiment Neutrokine-alpha and/or Neutrokine-alphaSV and/or Neutrokine-alpha Receptor polynucleotides, polypeptides, agonists, and/or antagonists are used to treat, prevent, and/or diagnose patients with cryptosporidiosis.

[0642] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and baeterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Baeillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobaeter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenie E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria (e.g, Listeria monocytogenes), Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaeeae (e.g., Aeinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellaeea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptoeoccus pneumoniae and Group B Streptococcus). These bacterial or fungal families can eause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokine alpha polynucleotides, polypeptides, or agonists thereof are

used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

[0643] Moreover, parasitic agents causing disease or symptoms that can be treated by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, inelude, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokine alpha polynucleotides, polypeptides, or agonists thereof are used to treat, prevent, and/or diagnose malaria.

[0644] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose inner ear infection (such as, for example, otitis media), as well as other infections characterized by infection with *Streptococcus pneumoniae* and other pathogenic organisms.

[0645] In a specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV antibodies) are used to treat or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, Neutrokine-alpha, and/or Neutrokine-alphaSVpolynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV antibodies) may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g.,

those diselosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition assoeiated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, multiple myeloma, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pheumocystis carnii.

[0646] Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or eonditions associated therewith: primary immunodeficiencies, immune-mediated thromboeytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[0647] Additionally, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions assoeiated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable mutliple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g, neonatal thromboeytopenia), and immune-mediated neutropenia), transplantation (e.g, cytamegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogammaglobulinemic neonates with risk factor for infection or morbidity), epilepsy (e.g, intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (e.g, decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[0648] Additional preferred embodiments of the invention inelude, but are not limited to, the use of Neutrokine-alpha and/or Neutrokine-alpha SV polypeptides, Neutrokine-alpha and/or Neutrokine-alpha SV polynucleotides, and functional agonists thereof, in the following applications:

[0649] Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and

human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to promote or enhance immunoglobulin class switching (e.g., to induce a B cell express an IgM antibody to class switch to a different immunoglobulin isotype such as IgG, IgA, or IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE, for instance, by the modulation of the rate or quantity of somatic hypermutation or by modulation of the process/mechanism of selection of B cells expressing mutated antibodies), and/or to increase an immune response. In a specific nonexclusive embodiment, Neutrokine-alpha polypeptides of the invention, and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgG. In another specific nonexclusive embodiment, Neutrokine-alpha polypeptides of the invention and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgA. In another specific nonexclusive embodiment, Neutrokine-alpha polypeptides of the invention and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgM.

[0650] Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741).

[0651] A vaccine adjuvant that enhances immune responsiveness to specific antigen. In a specific embodiment, the vaccine adjuvant is a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide described herein. In another specific embodiment, the vaccine adjuvant is a Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotide described herein (i.e., the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotide is a genetic vaccine adjuvant). As discussed herein, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides may be administered using techniques known in the art, including but not limited to, liposomal delivery, recombinant vector delivery, injection of naked DNA, and gene gun delivery.

[0652] An adjuvant to enhance tumor-specific immune responses.

[0653] An adjuvant to enhanee anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include, but are not limited to, virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhanee an immune response to the HIV gp120 antigen.

[0654] An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and baeteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: Vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphi, Meisseria meningitidis, Streptoeoccus pneumoniae, Group B streptococcus, Shigella spp., Enterotoxigenic Escherichia coli, Enterohemorrhagic E. coli, Borrelia burgdorferi, and Plasmodium (malaria).

[0655] An adjuvant to enhance anti-parasitie immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific

embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria).

[0656] As a stimulator of B cell responsiveness to pathogens.

[0657] As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0658] As an agent to induce production of higher affinity antibodies.

[0659] As an agent to induce class switching of B cells expressing IgM antibodies.

[0660] As an agent to induce class switching of aetivated B cells expressing IgM antibodies.

[0661] As an agent to increase serum immunoglobulin concentrations.

[0662] As an agent to accelerate recovery of immunocompromised individuals.

[0663] As an agent to boost immunoresponsiveness among aged populations.

[0664] As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a speeific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, eompositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

[0665] As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy. B cell immunodeficiencies that may be ameliorated or treated by administering the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, inelude, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspeeified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked

immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymie alymphoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficieney (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0666] As an agent to boost immunoresponsiveness among individuals having an acquired loss of B eell function. Conditions resulting in an acquired loss of B eell function that may be ameliorated or treated by administering the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, multiple myeloma and B cell chronic lymphocytic leukemia (CLL).

[0667] Patients with CLL and myeloma are at risk for increased infections. Thus, one aspect of the present invention provides for the use of Neutrokine alpha, Neutrokine alphaSV, anti-Neutrokine-alpha and or anti-Neutrokine alphaSV polynucleotides and/or polypeptides as an agent to boost immunoresponsiveness in CLL and myeloma patients.

[0668] As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, reeovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery, and recovery from burns.

[0669] As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides (in soluble, membrane-bound or transmembrane forms) or polynucleotides enhance

antigen presentation or antagonize antigen presentation in vitro or in vivo. Moreover, in related embodiments, this enhancement or antagonization of antigen presentation may be useful in anti-tumor treatment or to modulate the immune system.

[0670] As a mediator of mueosal immune responses. The expression of Neutrokine-alpha by monocytes and the responsiveness of B cells to this factor suggests that it may be involved in exchange of signals between B cells and monocytes or their differentiated progeny. This activity is in many ways analogous to the CD40-CD154 signaling between B cells and T eells. Neutrokine-alpha may therefore be an important regulator of T cell independent immune responses to environmental pathogens. In particular, the uneonventional B cell populations (CD5+) that are associated with mucosal sites and responsible for much of the innate immunity in humans may respond to Neutrokine-alpha thereby enhancing an individual's protective immune status.

[0671] As an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0672] As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refraetory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0673] As a B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

[0674] As a means of detecting B-lineage eells by virtue of its specificity. This application may require labeling the protein with biotin or other agents (e.g., as described herein) to afford a means of detection.

[0675] As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificieney.

[0676] As part of a B cell seleetion device the funetion of which is to isolate B cells from a heterogenous mixture of cell types. Neutrokine-alpha could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. A nonlimiting use of this selection would be to allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

[0677] As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

[0678] As a gene-based therapy for genetically inherited disorders resulting in immuno-ineompetence such as observed among SCID patients.

[0679] As an antigen for the generation of antibodies to inhibit or enhance Neutrokine-alpha mediated responses.

[0680] As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

[0681] As pretreatment of bone marrow samples prior to transplant. Sueh treatment would inerease B cell representation and thus accelerate recover.

[0682] As a means of regulating secreted cytokines that are elieited by Neutrokine-alpha.

[0683] Neutrokine-alpha or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists may be used to modulate IgE concentrations in vitro or in vivo.

[0684] Additionally, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

[0685] In a specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate seleetive IgA deficiency.

[0686] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate ataxia-telangieetasia.

[0687] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate common variable immunodeficiency.

[0688] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked agammaglobulinemia.

[0689] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate severe combined immunodeficiency (SCID).

[0690] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate Wiskott-Aldrich syndrome.

[0691] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked Ig deficiency with hyper IgM.

[0692] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists or antagonists (e.g., anti-Neutrokine-alpha antibodies) thereof, is administered to treat, prevent, and/or diagnose chronic myelogenous leukemia, acute myelogenous leukemia, leukemia, hystiocytic leukemia, monocytic leukemia (e.g., acute monocytic leukemia), leukemic reticulosis, Shilling Type monocytic leukemia, and/or other leukemias derived from monocytes and/or monocytic cells and/or tissues.

[0693] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukemoid reaction, as seen, for example, with tuberculosis.

[0694] In another specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukocytosis, monocytic leukopenia, monocytopenia, and/or monocytosis.

[0695] In a specific embodiment, Neutrokine-alpha, and Neutrokine-alphaSV polynucleotides or polypeptides of the invention, and/or anti-Neutrokine-alpha antibodies and/or agonists or antagonists thereof, are used to treat, prevent, detect, and/or diagnose primary B lymphocyte disorders and/or diseases, and/or conditions associated therewith. In one embodiment, such primary B lymphocyte disorders, diseases, and/or conditions are characterized by a complete or partial loss of humoral immunity. Primary B lymphocyte disorders, diseases, and/or conditions associated therewith that are characterized by a complete or partial loss of humoral immunity and that may be prevented, treated, detected

and/or diagnosed with compositions of the invention include, but are not limited to, X-Linked Agammaglobulinemia (XLA), severe combined immunodeficiency disease (SCID), and selective IgA deficiency.

[0696] In a preferred embodiment, Neutrokine-alpha and Neutrokine-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with any one or more of the various mucous membranes of the body. Such diseases or disorders include, but are not limited to, for example, mucositis, mucoclasis, mucocolitis, mucocutaneous leishmaniasis (such as, for example, American leishmaniasis, leishmaniasis americana, nasopharyngeal leishmaniasis, and New World leishmaniasis), mucocutaneous lymph node syndrome (for example, Kawasaki disease), mucoenteritis, mucoepidermoid carcinoma, mucoepidermoid tumor, mucoepithelial dysplasia, mucoid adenocarcinoma, mucoid degeneration, myxoid degeneration; myxomatous degeneration; myxomatosis, mucoid medial degeneration (for example, cystic medial necrosis), mucolipidosis (including, for example, mucolipidosis I, mucolipidosis II, mucolipidosis III, and mucolipidosis IV), mucolysis disorders, mucomembranous enteritis, mucoenteritis, mucopolysaccharidosis (such as, for example, type I mucopolysaccharidosis (i.e., Hurler's syndrome), type IS mucopolysaccharidosis (i.e., Scheie's syndrome or type V mucopolysaccharidosis), type II mucopolysaccharidosis (i.e., Hunter's syndrome), type III mucopolysaccharidosis (i.e., Sanfilippo's syndrome), type IV mucopolysaccharidosis (i.e., Morquio's syndrome), type VI mucopolysaccharidosis (i.e., Maroteaux-Lamy syndrome), type VII mucopolysaccharidosis (i.e, mucopolysaccharidosis due to beta-glucuronidase deficiency), and mucosulfatidosis), mucopolysaccharidiuria, mucopurulent conjunctivitis, mucopus, mucormycosis (i.e., zygomycosis), mucosal disease (i.e., bovine virus diarrhea), mucous colitis (such as, for example, mucocolitis and myxomembranous colitis), and mucoviscidosis (such as, for example, cystic fibrosis, cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis). In a highly preferred embodiment, Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose mucositis, especially as assoeiated with chemotherapy.

[0697] In a preferred embodiment, Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with sinusitis.

[0698] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, is osteomyelitis.

[0699] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, is endocarditis.

[0700] All of the above described applications as they may apply to veterinary medicine.

[0701] Antagonists of Neutrokine-alpha include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes, and Neutrokine-alpha polypeptides of the invention. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

[0702] A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens. Although our current data speaks direetly to the potential role of Neutrokine-alpha in B cell and monocyte related pathologies, it remains possible that other cell types may gain expression or responsiveness to Neutrokine-alpha. Thus, Neutrokine-alpha may, like CD40 and its ligand, be regulated by the status of the immune system and the microenvironment in which the cell is located.

[0703] A therapy for preventing the B cell proliferation and Ig seeretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and MS.

[0704] An inhibitor of graft versus host disease or transplant rejeetion.

[0705] A therapy for B cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphoeyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

[0706] A therapy for chronie hypergammaglobulinemeia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoelonalgammopathies, and plasmacytomas.

[0707] A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

[0708] A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

[0709] An immunosuppressive agent(s).

[0710] Neutrokine-alpha or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or antagonists may be used to modulate IgE concentrations in vitro or in vivo.

[0711] In another embodiment, administration of Neutrokine-alpha or Neutrokine-alphaSV polypeptides or polynucleotides of the invention, or antagonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

[0712] An inhibitor of signaling pathways involving ERK1, COX2 and Cyelin D2 which have been associated with Neutrokine-alpha induced B cell activation.

[0713] The above-recited applications have uses in a wide variety of hosts. Sueh hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0714] The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0715] The antagonists may be employed for instance to inhibit Neutrokine-alpha-mediated and/or Neutrokine-alphaSV-mediated chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronie inflammatory and infective diseases. Examples of auto-immune

diseases include multiple sclerosis, and insulin-dependent diabetes. The antagonists may also be employed to treat, prevent, and/or diagnose infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat, prevent, and/or diagnose idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the present invention. The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall. The antagonists may also be employed to treat, prevent, and/or diagnose histamine-mediated allergic reaetions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated. The antagonists may also be employed to treat, prevent, and/or diagnose chronic and aeute inflammation by preventing the attraetion of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. Antagonists may also be employed to treat, prevent, and/or diagnose rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients. Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies. The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory eytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by Neutrokine-alpha and/or Neutrokine-alphaSV. The antagonists may also be employed to treat, prevent, and/or diagnose eases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome. The antagonists may also be employed to treat, prevent, and/or diagnose asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat, prevent, and/or diagnose subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung. The antagonists may also be

employed to treat, prevent, and/or diagnose lymphomas (e.g., one or more of the extensive, but not limiting, list of lymphomas provided herein).

[0716] All of the above described applications as they may apply to veterinary medicine. Moreover, all applications described herein may also apply to veterinary medicine.

[0717] Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may be used to treat, prevent, and/or diagnose various immune system-related disorders and/or conditions associated with these disorders, in mammals, preferably humans. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof that can inhibit an immune response, particularly the proliferation of B cells and/or the production of immunoglobulins, may be an effective therapy in treating and/or preventing autoimmune disorders. Thus, in preferred embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV antagonists of the invention (e.g., polypeptide fragments of Neutrokine-alpha and/or Neutrokine-alphaSV and anti-Neutrokine-alpha antibodies) are used to treat, prevent, and/or diagnose an autoimmune disorder.

[0718] Autoimmune disorders and conditions associated with these disorders that may be treated, prevented, and/or diagnosed with the Neutrokine-alpha polynucleotides, polypeptides, and/or antagonists of the invention (e.g,. anti-Neutrokine-alpha antibodies), include, but are not limited to, autoimmune hemolytic anemia, autoimmune neutropenia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g, IgA nephropathy), dense deposit disease, Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, gluten sensitive enetropathy, insulin dependent diabetes mellitis, discoid lupus, and autoimmune inflammatory eye disease.

[0719] Additional autoimmune disorders (that are highly probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention inelude, but are not limited to, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis) (often characterized, e.g., by eell-mediated and humoral thyroid cytotoxicity), systemic lupus erhythematosus (often characterized, e.g., by circulating and loeally generated immune complexes), Goodpasture's syndrome (often characterized, e.g., by anti-basement membrane antibodies), Pemphigus (often characterized, e.g., by epidermal acantholytic antibodies), Receptor autoimmunities such as, for example, (a) Graves' Disease (often characterized, e.g., by TSH reeeptor antibodies), (b) Myasthenia Gravis (often characterized, e.g., by acetylcholine receptor antibodies), and (c) insulin resistance (often characterized, e.g., by insulin receptor antibodies), autoimmune hemolytic anemia (often characeterized, e.g., by phagocytosis of antibody-sensitized RBCs), autoimmune thrombocytopenie purpura (often characterized, e.g., by phagocytosis of antibody-sensitized platelets.

[0720] Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention inelude, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), sehleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often charaeterized, e.g., by antibodies to extractable nuelear antigens (e.g., ribonucleoprotein)), polymyositis/dermatomyositis (often eharacterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often charaeterized, e.g., by glomerular basement membrane antibodies or immune eomplexes) such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid (often charaeterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often charaeterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes millitus (often charaeterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

[0721] Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitchondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiotomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), inflammatory myopathies, and many other inflammatory, granulamatous, degenerative, and atrophic disorders.

[0722] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV.

[0723] In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0724] In a specific preferred embodiment, lupus is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0725] In a specific preferred embodiment, Sjögren's Syndrome is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0726] In a specific preferred embodiment, AIDS is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0727] In a specific preferred embodiment, HIV infection is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0728] In a specific preferred embodiment, nephritis associated with lupus is treated, prevented, and/or diagnosed using anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies and/or other antagonist of the invention.

[0729] In a specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) are used to treat or prevent systemic lupus erythematosus and/or diseases, disorders or conditions associated therewith. Lupus-associated diseases, disorders, or conditions that may be treated or prevented with Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or antagonists of the invention, include, but are not limited to, hematologic disorders (e.g., hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia), immunologic disorders (e.g., anti-DNA antibodies, and anti-Sm antibodies), rashes, photosensitivity, oral ulcers, arthritis, fever, fatigue, weight loss, serositis (e.g., pleuritus (pleuricy)), renal disorders (e.g., nephritis), neurological disorders (e.g., seizures, peripheral neuropathy, CNS related disorders), gastroinstestinal disorders, Raynaud phenomenon, and pericarditis. In a preferred embodiment, the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) are used to treat or prevent renal disorders associated with systemic lupus erythematosus. In a most preferred embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) are used to treat or prevent nephritis associated with systemic lupus erythematosus.

[0730] Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

[0731] Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD) and/or conditions associated therewith. Organ rejection occurs by host immune cell destruction

of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

[0732] Similarly, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to modulate inflammation. For example, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

[0733] In a specific embodiment, anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflammation.

[0734] In a specific embodiment, anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflamatory disorders.

[0735] In another specific embodiment, anti-Neutrokine-alpha antibodies and/or anti-Neutrokine-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose allergy and/or hypersensitivity.

[0736] In another embodiment, therapeutic or pharmaceutical compositions of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof) are administered to an animal to

treat, prevent or ameliorate ischemia and arteriosclerosis. Examples of such disorders include, but are not limited to, reperfusion damage (*e.g.*, in the heart and/or brain) and cardiac hypertrophy.

[0737] Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof, may also be used to modulate blood clotting and to treat or prevent blood clotting disorders, such as, for example, antibody-mediated thrombosis (i.e., antiphospholipid antibody syndrome (APS)). For example, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof, may inhibit the proliferation and differentiation of cells involved in producing anticardiolipin antibodies. These compositions of the invention can be used to treat, prevent, and/or diagnose, thrombotic related events including, but not limited to, stroke (and recurrent stroke), heart attack, deep vein thrombosis, pulmonary embolism, myocardial infarction, coronary artery disease (e.g,. antibody –mediated coronary artery disease), thrombosis, graft reocclusion following cardiovascular surgery (e.g,. coronary arterial bypass grafts, recurrent fetal loss, and recurrent cardiovascular thromboembolic events.

[0738] Antibodies against Neutrokine-alpha and/or Neutrokine-alphaSV may be employed to bind to and inhibit Neutrokine-alpha and/or Neutrokine-alphaSV aetivity to treat, prevent, and/or diagnose ARDS, by preventing infiltration of neutrophils into the lung after injury. The agonists and antagonists of the instant may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described hereinafter.

[0739] Neutrokine-alpha and/or Neutrokine-alphaSV and/or Neutrokine-alpha receptor polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose diseases and disorders of the pulmonary system (e.g., bronchi such as, for example, sinopulmonary and bronchial infections and conditions associated with such diseases and disorders and other respiratory diseases and disorders. In specific embodiments, such diseases and disorders include, but are not limited to, bronchial adenoma, bronehial asthma, pneumonia (such as, e.g., bronchial pneumonia, bronchopneumonia, and tuberculous bronchopneumonia), chronic obstructive pulmonary disease (COPD), bronchial polyps, bronchiectasia (such as, e.g., bronchiectasia sicca, cylindrical bronchiectasis, and saccular bronchiectasis), bronchiolar adenocarcinoma, bronchiolar carcinoma, bronchiolitis (such as, e.g., exudative

bronchiolitis, bronchiolitis fibrosa obliterans, and proliferative bronchiolitis), bronchiolo-alveolar carcinoma, bronchitic asthma, bronchitis (such as, e.g., asthmatic bronchitis, Castellani's bronchitis, chronic bronchitis, croupous bronchitis, fibrinous bronchitis, hemorrhagic bronchitis, infectious avian bronchitis, obliterative bronchitis, plastic bronchitis, pseudomembranous bronchitis, putrid bronchitis, and verminous bronchitis), bronchoecntric granulomatosis, bronchocdema, bronchocsophageal fistula, bronchogenic carcinoma, bronchogenic cyst, broncholithiasis, bronchomalacia, bronchomycosis (such as, e.g., bronchopulmonary aspergillosis), bronchopulmonary spirochetosis, hemorrhagic bronchitis, bronchorrhea, bronchospasm, bronchostaxis, bronchostenosis, Biot's respiration, bronchial respiration, Kussmaul respiration, Kussmaul-Kien respiration, respiratory acidosis, respiratory alkalosis, respiratory distress syndrome of the newborn, respiratory insufficiency, respiratory scleroma, respiratory syncytial virus, and the like.

[0740] In a specific embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose chronic obstructive pulmonary disease (COPD).

[0741] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose fibroses and conditions associated with fibroses, such as, for example, but not limited to, cystic fibrosis (including such fibroses as cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis), endomyocardial fibrosis, idiopathic retroperitoneal fibrosis, leptomeningeal fibrosis, mediastinal fibrosis, nodular subepidermal fibrosis, pericentral fibrosis, perimuscular fibrosis, pipestem fibrosis, replacement fibrosis, subadventitial fibrosis, and Symmers' clay pipestem fibrosis.

[0742] The TNF family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (D.V. Goeddel *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol. 51*:597- 609 (1986), Cold Spring Harbor; B. Beutler and A. Cerami, *Annu. Rev. Biochem. 57*:505-518 (1988); L.J. Old, *Sci. Am. 258*:59-75 (1988); W. Fiers, *FEBS Lett. 285*:199-224 (1991)). The TNF-family ligands, including Neutrokine-alpha

and/or Neutrokine-alphaSV of the present invention, induce sueh various eellular responses by binding to TNF-family receptors. Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides are believed to elicit a potent cellular response including any genotypic, phenotypic, and/or morphologie change to the cell, cell line, tissue, tissue culture or patient. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with inereased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral B and/or T lymphocytes of the immune system, and its disregulation ean lead to a number of different pathogenic processes (J.C. Ameisen, *AIDS 8*:1197-1213 (1994); P.H. Krammer *et al., Curr. Opin. Immunol. 6*:279-289 (1994)).

[0743] Diseases assoeiated with inereased cell survival, or the inhibition of apoptosis that may be diagnosed, treated, or prevented with the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, and agonists and antagonists thereof, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to, colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal eancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate caneer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis); viral infections (such as herpes viruses, pox viruses and adenoviruses); inflammation; graft vs. host disease; acute graft rejection and chronic graft rejeetion. Thus, in preferred embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose autoimmune diseases and/or inhibit the growth, progression, and/or metastasis of cancers, including, but not limited to, those eancers diselosed herein, such as, for example, lymphocytic leukemias (including, for example, MLL and chronic lymphocytic leukemia (CLL)) and follicular lymphomas. In another embodiment Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention are used to activate, differentiate or proliferate cancerous cells or tissue (e.g., B cell lineage related cancers (e.g., CLL and MLL), lymphocytic

leukemia, or lymphoma) and thereby render the cells more vulnerable to cancer therapy (e.g., chemotherapy or radiation therapy).

[0744] Moreover, in other embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention or agonists or antagonists thereof, are used to inhibit the growth, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocareinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0745] In specific embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of multiple myeloma. In even more specific embodiments, radiolabeled Neutrokine-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokine alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of multiple myeloma. In

particular embodiments, the radiolabeled Neutrokine-alpha polypeptides are radiolabeled with an ^{131}I radioisotope.

[0746] In specific embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of non-Hodgkin's lymphoma. In even more specific embodiments, radiolabeled Neutrokine-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokine alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of non-Hodgkin's lymphoma. In particular embodiments, the radiolabeled Neutrokine-alpha polypeptides are radiolabeled with an ^{131}I radioisotope.

[0747] In specific embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of chronic lymphocytic leukemia (CLL). In even more specific embodiments, radiolabeled Neutrokine-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokine alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of CLL. In particular embodiments, the radiolabeled Neutrokine-alpha polypeptides are radiolabeled with an ^{131}I radioisotope.

[0748] Diseases associated with increased apoptosis apoptosis that may be diagnosed, treated, or prevented with the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, and agonists and antagonists thereof,include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-indueed liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. Thus, in preferred embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose the diseases and disorders listed above.

[0749] In preferred embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha antibodies) inhibit the growth of human histiocytic lymphoma U-937 cells in a dose-dependent manner. In additional preferred embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha antibodies) inhibit the growth of PC-3 cells, HT-29 cells, HeLa cells, MCF-7 cells, and A293 cells. In highly preferred embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV polynueleotides or polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokine-alpha antibodies) are used to inhibit growth, progression, and/or metastasis of prostate cancer, colon cancer, cervical careinoma, and breast earcinoma.

[0750] Thus, in additional preferred embodiments, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses a Neutrokine-alpha and/or Neutrokine-alphaSV receptor an effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV, or an agonist or antagonist thereof, capable of increasing or decreasing Neutrokine-alpha and/or Neutrokine-alphaSV mediated signaling. Preferably, Neutrokine-alpha and/or Neutrokine-alphaSV mediated signaling is increased or decreased to treat, prevent, and/or diagnose a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited. An agonist or antagonist can include soluble forms of Neutrokine-alpha and/or Neutrokine-alphaSV and monoclonal antibodies directed against the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide.

[0751] In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the Neutrokine-alpha and/or Neutrokine-alphaSV receptor an effective amount of an agonist or antagonist capable of increasing or decreasing Neutrokine-alpha and/or Neutrokine-alphaSV mediated signaling. Preferably, Neutrokine-alpha and/or Neutrokine-alphaSV mediated signaling is increased or decreasd to treat, prevent, and/or diagnose a disease wherein increased apoptosis or NF-kappaB expression is exhibited. An agonist or antagonist can include soluble forms of Neutrokine-alpha and/or Neutrokine-alphaSV and monoclonal antibodies directed against the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide.

[0752] Because Neutrokine-alpha and/or Neutrokine-alphaSV belong to the TNF superfamily, the polypeptides should also modulate angiogenesis. In addition, since Neutrokine-alpha and/or Neutrokine-alphaSV inhibit immune cell functions, the polypeptides will have a wide range of anti-inflammatory activities. Neutrokine-alpha and/or Neutrokine-alphaSV may be employed as an anti-neovascularizing agent to treat, prevent, and/or diagnose solid tumors by stimulating the invasion and activation of host defense cells, e.g., cytotoxie T cells and macrophages and by inhibiting the angiogenesis of tumors. Those of skill in the art will recognize other non-cancer indications where blood vessel proliferation is not wanted. They may also be employed to enhance host defenses against resistant chronie and acute infections, for example, myobacterial infections via the attraction and activation of microbicidal leukocytes. Neutrokine-alpha and/or Neutrokine-alphaSV may also be employed to inhibit T-cell proliferation by the inhibition of IL-2 biosynthesis for the treatment of T-cell mediated auto-immune diseases and lymphocytic leukemias (ineluding, for example, chronic lymphocytic leukemia (CLL)). Neutrokine-alpha and/or Neutrokine-alphaSV may also be employed to stimulate wound healing, both via the reeruitment of debris clearing and connective tissue promoting inflammatory cells. In this same manner, Neutrokine-alpha and/or Neutrokine-alphaSV may also be employed to treat, prevent, and/or diagnose other fibrotic disorders, including liver cirrhosis, osteoarthritis and pulmonary fibrosis. Neutrokine-alpha and/or Neutrokine-alphaSV also increases the presence of eosinophils that have the distinetive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis. It may also be employed to regulate hematopoiesis, by regulating the activation and differentiation of various hematopoietic progenitor eells, for example, to release mature leukocytes from the bone marrow following chemotherapy, i.e., in stem cell mobilization. Neutrokine-alpha and/or Neutrokine-alphaSV may also be employed to treat, prevent, and/or diagnose sepsis.

[0753] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast caneer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell

carcinoma of the lung, small eell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infeetion, hepatitis infection (e.g., HAV, HBV, HCV, ete.), Helicobacter pylori infection, invasive Staphylococcia, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.)), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., aeute and ehronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune eomplex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, ete.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

[0754] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting angiogenesis, wound healing (e.g., wounds, burns, and bone fractures). Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as an adjuvant to enhance immune responsiveness to specific antigen, anti-viral immune responses.

[0755] More generally, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or redueing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and immunocompromised individuals. Alternatively, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists

thereof are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

[0756] Preferably, treatment using Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, and/or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV (e.g., anti-Neutrokine-alpha antibody), could either be by administering an effective amount of Neutrokine-alpha, and/or Neutrokine-alphaSV polypeptide of the invention, or agonist or antagonist thereof, to the patient, or by removing cells from the patient, supplying the cells with Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, as further diseussed herein, the Neutrokine-alpha, and/or Neutrokine-alphaSV polypeptide or polynueleotide can be used as an adjuvant in a vaceine to raise an immune response against infeetious disease.

Formulations and Administration

[0757] The Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide composition (preferably containing a polypeptide which is a soluble form of the Neutrokine-alpha and/or Neutrokine-alphaSV extracellular domains) will be formulated and dosed in a fashion consistent with good medical practice, taking into aceount the clinical condition of the individual patient (especially the side effects of treatment with Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide alone), the site of delivery of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide composition, the method of administration, the scheduling of administration, and other faetors known to practitioners. The "effective amount" of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide for purposes herein is thus determined by such considerations.

[0758] As a general proposition, the total pharmaceutically effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide administered parenterally per dose will be in the range of about 1 microgram/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day.

[0759] In another embodiment, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide of the invention is administered to a human at a dose betweeen 0.0001 and 0.045 mg/kg/day, preferably, at a dose between 0.0045 and 0.045 mg/kg/day, and more preferably, at a dose of about 45 microgram/kg/day in humans; and at a dose of about 3 mg/kg/day in mice.

[0760] If given continuously, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is typically administered at a dose rate of about 1 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0761] The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0762] In a specific embodiment, the total pharmaceutically effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide administered parenterally per dose will be in the range of about 0.1 microgram/kg/day to 45 micrograms/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.1 microgram/kg/day, and most preferably for humans between about 0.01 and 50 micrograms/kg/day for the protein. Neutrokine-alpha and/or Neutrokine-alphaSV may be administered as a continuous infusion, multiple dicreet injections per day (e.g., three or more times daily, or twice daily), single injection per day, or as discreet injections given intermitently (e.g., twice daily, once daily, every other day, twice weekly, weekly, biweekly, monthly, bimonthly, and quarterly). If given continuously, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is typically administered at a dose rate of about 0.001 to 10 microgram/kg/ hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump.

[0763] Effective dosages of the compositions of the present invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Such determination is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0764] Bioexposure of an organism to Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide during therapy may also play an important role in determining a therapeutically and/or pharmacologically effective dosing regime. Variations of dosing such as repeated administrations of a relatively low dose of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide for a relatively long period of time may have an effect which is therapeutically and/or pharmaeologically distinguishable from that achieved with repeated administrations of a relatively high dose of Neutrokine-alpha and/or Neutrokine-alphaSV for a relatively short period of time. See, for instance, the serum immunoglobulin level experiments presented in Example 6.

[0765] Using the equivalent surface area dosage conversion factors supplied by Freireich, E. J., et al. (*Cancer Chemotherapy Reports* 50(4):219-44 (1966)), one of ordinary skill in the art is able to conveniently convert data obtained from the use of Neutrokine-alpha and/or Neutrokine-alphaSV in a given experimental system into an aceurate estimation of a pharmaceutically effective amount of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide to be administered per dose in another experimental system. Experimental data obtained through the administration of Neutrokine-alpha in miee (see, for instanee, Example 6) may converted through the conversion factors supplied by Freireich, et al., to accurate estimates of pharmaceutically effective doses of Neutrokine-alpha in rat, monkey, dog, and human. The following conversion table (Table III) is a summary of the data provided by Freireich, et al. Table III gives approximate factors for converting doses expressed in terms of mg/kg from one speeies to an equivalent surface area dose expressed as mg/kg in another species tabulated.

Table III. Equivalent Surface Area Dosage Conversion Factors.

			--TO--		
	Mouse	Rat	Monkey	Dog	Human
--FROM--	(20g)	(150g)	(3.5kg)	(8kg)	(60kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	5/3	1	1/2
Human	12	7	3	2	1

[0766] Thus, for example, using the conversion factors provided in Table III, a dose of 50 mg/kg in the mouse converts to an appropriate dose of 12.5 mg/kg in the monkey because (50 mg/kg) x (1/4) = 12.5 mg/kg. As an additional example, doses of 0.02, 0.08, 0.8, 2, and 8 mg/kg in the mouse equate to effect doses of 1.667 micrograms/kg, 6.67 micrograms/kg, 66.7 micrograms/kg, 166.7 micrograms/kg, and 0.667 mg/kg, respeetively, in the human.

[0767] Pharmaceutical eompositions containing Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention may be administered orally, rectally, parenterally, subcutaneously, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal pateh), bucally, or as an oral or nasal spray (e.g., via inhalation of a vapor or powder). In one embodiment, "pharmaceutically aeceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutieally aeceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. Nonlimiting examples of suitable pharmaeeutical carriers according to this embodiment are provided in "Remington's Pharmaceutical Sciences" by E.W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can be employed as liquid carriers, partieularly for injectable solutions. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0768] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0769] In a preferred embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered subcutaneously.

[0770] In another preferred embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered intravenously.

[0771] Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0772] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers 22*:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0773] In a preferred embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific preferred embodiments the Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado). In other specific embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention are formulated using the ProLease® sustained relase sytem available from Alkermes, Inc. (Cambridge, MA).

[0774] Examples of biodegradable polymers which can be used in the formulation of Neutrokine-alpha and/or Neutrokine-alphaSV compositions, include but are not limited to,

polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of erystallization and are more hydrophobic. These polymers and eopolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polyeaprolactones, and eopolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specifie preferred embodiments, the biodegradable polymers which can be used in the formulation of Neutrokine-alpha and/or Neutrokine-alphaSV compositions are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug Neutrokine-alpha and/or Neutrokine-alphaSV release profile (See, e.g., Ravivarapu et al., Journal of Pharmaceutical Sciences 89:732-741 (2000), which is hereby incorporated by refernce in its entirety).

[0775] It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limted to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alchohols, dils, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alchohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and

triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

[0776] Additionally, formulations comprising Neutrokine-alpha and/or Neutrokine-alphaSV compositions and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglyeerides, other like hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modifieation agents inelude, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl eitrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triaeetate, di(n-butyl) sebecate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C.sub.6 -C.sub.12 alkanols, 2-ethoxyethanol, and the like. The release rate modification agent may be used singly or in combination with other sueh agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to, sugars such as suerose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

[0777] In specific preferred embodiments the Neutrokine-alpha and/or Neutrokine-alphaSV compositions of the invention are formulated using the BEMA™ BioErodible Mucoadhesive System, MCA™ MucoCutaneous Absorption System, SMP™ Solvent MicroParticle System, or BCP™ BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

[0778] Sustained-release compositions also inelude liposomally entrapped compositions of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide my be prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Aead. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide therapy.

[0779] In another embodiment sustained release compositions of the invention include crystal formulations known in the art.

[0780] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0781] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0782] For parenteral administration, in one embodiment, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0783] Generally, the formulations are prepared by contacting the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a

solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0784] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, sucrose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; preservatives, such as cresol, phenol, chlorobutanol, benzyl alcohol and parabens, and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0785] The Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is typically formulated in such vehicles at a concentration of about 0.001 mg/ml to 100 mg/ml, or 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml or 1-10 mg/ml, at a pH of about 3 to 10, or 3 to 8, more preferably 5-8, most preferably 6-7. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide salts.

[0786] Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0787] Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide solution, and the

resulting mixture is lyophilized. The infusion solution is prepared by reeonstituting the lyophilized Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide using bacteriostatic Water-for-Injection.

[0788] Alternatively, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is stored in single dose containers in lyophilized form. The infusion seleetion is reconstituted using a sterile carrier for injection.

[0789] A composition of the invention may comprise Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide that is radiolabeled, for example, with radioactive isotopes of iodine. Compositions comprising iodinated forms of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or fragments or variants thereof, may also comprise radioprotectants and plasma expanders such as sodium ascorbate, gentran-40, and glyeerol. In specific embodiments, compositions of the invention comprising iodinated forms of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or fragments or variants are formulated in 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Genetran-40. The above described compositions may be used as pharmaceutical compositions.

[0790] In specific embodiments, a composition of the invention comprises, at least 1 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, at least 2 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, at least 3 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium eitrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In speeific embodiments, a composition of the invention comprises, at least 4 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In particular embodiments, a composition of the invention comprises, about 4.6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v)

Gentran-40. The above described compositions may be used as pharmaceutical compositions.

[0791] In specific embodiments, a composition of the invention comprises, about between 0.1 mg/mL and 20mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specifie embodiments, a composition of the invention comprises, between 1mg/mL and 10mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, between 2mg/mL and 8mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, between 3mg/mL and 6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. The above described compositions may be used as pharmaceutical compositions.

[0792] The invention also provides a pharmaceutical pack or kit comprising one or more eontainers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally, associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biologieal produets, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

[0793] The eompositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a speeific embodiment, compositions of the invention are administered in combination with alum. In another speeific embodiment, compositions of the invention are administered in eombination with QS-21. Further adjuvants that may be administered with the compositions of the invention include, but are not limited to, Monophosphoryl

lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the compositions of the invention inelude, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™. Combinations may be administered either conеomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0794] In a specific embodiment, compositions of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention, Neutrokine-alpha and/or Neutrokine-alphaSV fragments and variants, and anti-Neutokine-alpha and/or anti-Netrokine-alphaSV antibodies) may be administered to patients as vaccine adjuvants. In a further specific embodiment, compositions of the invention may be administered as vaccine adjuvants to patients suffering from an immune-deficiency. In a further specific embodiment, compositions of the invention may be administered as vaecine adjuvants to patients suffering from HIV.

[0795] In a specific embodiment, compositions of the invention may be used to increase or enhanee antigen-specific antibody responses to standard and experimental vaccines. In a specific embodiment, compositions of the invention may be used to enhance seroconversion in patients treated with standard and experimental vaccines. In another specific embodiment, compositions of the invention may be used to inerease the number of unique epitopes recognized by antibodies elicited by standard and experimental vaccination.

[0796] In another specific embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or eondition associated therewith. In one embodiment,

compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, compositions of the invention are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0797] The compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0798] In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), TR6 (International

Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12.

[0799] In another embodiment, the compositions of the invention are invention are administered in combination with Neutrokine-alpha receptors and/or Neurtokine-alpha SV receptors (e.g., TACI and BCMA) In preferred in emodiments the Neutrokine-alpha receptors and/or Neurtokine-alpha SV receptors are soluble. In other preferred embodiments the the Neutrokine-alpha receptors and/or Neurtokine-alpha SV receptors are fused to the FC region of an immunoglobulon molecule (c.g, amino acid residues 1-154 of TACI (GenBank accesion number AAC51790), or 1-48 of BCMA (GenBank accession numbcr NP_001183) fused to the Fc region of an IgG molecule.

[0800] In a prefcrred embodiment, the compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), bioloigically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g,. agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g, agonistic or antagonistic antibodies).

[0801] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thercof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloprotcinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogcn Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0802] Lighter "d group" transition mctals includc, for cxamplc, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal

species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0803] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0804] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0805] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet faetor 4; protamine sulphate; sulphated ehitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglyean Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids sueh as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, ineluding for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al.,

Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

[0806] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0807] Anti-angiogenic agents that may be administered in combination with the eompositions of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial eell-extraeellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial eells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven , CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Bioteeh, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the

compositions of the invention include, but are not limited to, EMD-121974 (Merck KcgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that aet by directly antagonizing or inhibiting angiogenesis indueers and which may be administered in eombination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genenteeh, S. San Franeisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis whieh may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roehe, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

[0808] In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

[0809] In a particular embodiment, the use of compositions of the invention in eombination with anti-angiogenic agents is eontemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

[0810] In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of strokes.

[0811] In another embodiment, compositions of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another speeific embodiment, compositions of the invention are administered in combination with warfarin

and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

[0812] In another embodiment, compositions of the invention are administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In specific embodiments, the polynucleotides of the invention are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0813] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat, prevent, and/or diagnose AIDS and/or to treat, prevent, and/or diagnose HIV infection.

[0814] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™

(stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat AIDS and/or to prevent or treat HIV infeetion.

[0815] Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β-L-FD4C and β-L-FddC (WO 98/17281).

[0816] Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predeeessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A

(naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

[0817] Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myres Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Welcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

[0818] Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain whieh binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

[0819] Additional antiretroviral agents include fusion inhibitors/ehemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists inelude CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES , NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine recpetor agonists such as RANTES, SDF-1, MIP-1α, MIP-1β, etc., may also inhibit fusion.

[0820] Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinie (DFQA) acids; L-chicoric acid (a dicaffeoyltartarie (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonueleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

[0821] Additional antiretroviral agents include hydroxyurea-like eompunds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate

dehydrogenase (IMPDH) inhibitors sucha as VX-497 (Vertex); and myvopholic acids such as CellCept (myeophenolate mofetil; Roche).

[0822] Additional antiretroviral agents inelude inhibitors of viral integrase, inhibitors of viral genome nuelear translocation such as arylene bis(methylketone) eompounds; inhibitors of HIV entry sueh as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble eomplexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors sueh as dithiane compounds; targets of HIV Tat and Rev; and pharmaeoenhancers such as ABT-378.

[0823] Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1α, MIP-1β, SDF-1α, IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN-α2a; antagonists of TNFs, NFκB, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyelosporin and prednisone; vaceines sueh as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branehed synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaceines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetieally modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al., PNAS 94*:11567-72 (1997); Chen *et al., Nat. Med. 3*:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF-α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α-naphthoflavone (WO 98/30213); and antioxidants such as γ-L-glutamyl-L-cysteine ethyl ester (γ-GCE; WO 99/56764).

[0824] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistie agents that may be administered in combination with the compositions of the invention, include, but are

not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylaetically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specifie embodiment, eompositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylaetically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with

LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0825] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0826] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention inelude, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamyein, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0827] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the eompositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the funetion of responding T cells. Other immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamyein, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685).

[0828] In specific embodiments, compositions of the invention are administered in combination with immunosuppressants. Immunosuppressant preparations that may be administered with the compositions of the invention include, but are not limited to, ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate motefil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrxate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™

(sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0829] In a preferred embodiment, the compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV. In a another specific embodiment, compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV.

[0830] In a preferred embodiment, the compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0831] In a preferred embodiment, the compositions of the invention are administered in combination with an NSAID.

[0832] In a nonexclusive embodiment, the compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasermin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone

mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0833] In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.), SCIO-469 (p38 kinase inhibitor from Scios, Inc), and/or ASLERA™ (prasterone, dehydroepiandrosterone, GL701) from Genelabs Technologies Inc.

[0834] In a preferred embodiment, the compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California), and prednisolone.

[0835] In a more preferred embodiment, the compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the compositions of the invention are administered in combination with methotrexate. In another embodiment, the compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the compositions of the invention are administered in combination ENBREL™. In another embodiment, the compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In

another embodiment, the compositions of the invention arc administered in combination with ENBREL™, and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specfic embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specfic embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0836] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, eompositions of the invention are administered in eombination with intravenous immune globulin preparations in transplantation thcrapy (e.g., bone marrow transplant).

[0837] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. . Anti-inflammatory agents that may be administered with the eompositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic aeid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylie acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0838] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-CD4 antibody. In one embodiment, eoadministration of the compositions of the invention with anti-CD4 antibody is envisoned for treatment of rheumatoid arthritis.

[0839] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-IL-15 antibody. In one embodiment, coadministration

of the compositions of the invention with anti-IL-15 antibody is envisoned for treatment of rheumatoid arthritis.

[0840] In specific embodiments, the compositions of the invention are administered alone or in combination with CTLA4-Ig and LEA29Y. In one embodiment, coadministration of the compositions of the invention with CTLA4-Ig and LEA29Y is envisoned for treatment of rheumatoid arthritis.

[0841] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-IL-6 Receptor antibody. In one embodiment, coadministration of the compositions of the invention with anti-IL-6 Receptor antibody is envisoned for treatment of rheumatoid arthritis.

[0842] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-C5 (complement component) antibody. In one embodiment, coadministration of the compositions of the invention with anti-C5 antibody is envisoned for treatment of rheumatoid arthritis.

[0843] In specific embodiments, the compositions of the invention are administered alone or in combination with complement cascade inhibitors. Complement cascade inhibitors include, but are notl imited to, anti-properdin antibodies (Gliatech); TP-10, a recombinant soluble type I complement receptor (AVANT Immunotheragenetics Inc.); Pexelizmab, a Complement C5 inhibitor (Alexion Pharmaceuticals Inc.); and 5G1.1, a monoclonal antibody that prevents cleavage of complement component C5 into its pro-inflammatory components. In one embodiment, coadministration of the compositions of the invention with complement cascade inhibitors are is envisoned for treatment of Inflammation, Rheumatoid arthritis, and/or cardiovascular disorders.

[0844] In another embodiment, compostions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone,

cstramustine phosphate sodium, ethinyl estradiol, estradiol, mcgestrol acetate, methyltestostcronc, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard dcrivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotcpa); steroids and combinations (e.g., bethamcthasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0845] In a specifie embodimcnt, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisonc. In a speeifie embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the eomponents of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab (anti-CD20 antibody from Coulter Pharmaceuticals, San Franciseo, CA). In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any eombination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Tositumomab may optionally bc associated with 131I. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxie prodrugs.

[0846] In another specifie embodiment, thc compositions of the invention arc administercd in combination Zevalin™. In a further embodiment, eompositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisonc. Zevalin™ may bc associatcd with one or more radisotopcs. Particularly preferred isotopes are ^{90}Y and ^{111}In.

[0847] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In another embodiment, compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the compositions of the invention are administered in combination with IL4 and IL10. Both IL4 and IL10 have been observed by the inventors to enhance Neutrokine-alpha mediated B cell proliferation.

[0848] In vitro, IFN gamma and IL-10 have each been observed by the inventors to enhance cell surface expression of Neutrokine-alpha in monocytes and macrophages (macrophages were obtained by culturing primary monocytes with 20ng/mL of M-CSF for 12-15 days), whereas IL-4 treatment decreased cell surface expression of Neutrokine-alpha in monocytes and macrophages. IL-4 administered with IL-10 resulted in a complete inhibition of the IL-10 induced cell surface expression of Neutrokine-alpha. IL-4 administered with IFN-gamma resulted in inereased cell-surface expression of Neutrokine-alpha. Treatment of macrophages with IFN-gamma and IL-10 resulted in a 3 fold increase of soluble (active) Neutrokine–alpha released into the culture medium compared to untreated macrophages.

[0849] In an additional embodiment, the compositions of the invention are administered with a chemokine. In another embodiment, the compositions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the compositions of the invention are administered with ehemokine beta-8.

[0850] In an additional embodiment, the compositions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4

receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0851] In an additional embodiment, the compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0852] In an additional embodiment, the compositions of the invention are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0853] Additionally, the compositions of the invention may be administered alone or in combination with other therapeutic regimens, including but not limited to, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

Agonists and Antagonists - Assays and Molecules

[0854] The invention also provides a method of screening compounds to identify those which enhance or block the action of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide on cells, such as its interaction with Neutrokine-alpha and/or Neutrokine-alphaSV binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of Neutrokine-alpha and/or Neutrokine-alphaSV or which functions in a manner similar to Neutrokine-alpha and/or Neutrokine-alphaSV while antagonists decrease or eliminate such functions.

[0855] In another embodiment, the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokine-alpha and/or Neutrokine-alphaSV. The

preparation is incubated with labeled Neutrokine-alpha and/or Neutrokine-alphaSV and complexes of Neutrokine-alpha and/or Neutrokine-alphaSV bound to the receptor or other binding protein are isolated and characterized aceording to routine methods known in the art. Alternatively, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and eharaeterized aecording to routine methods.

[0856] In the assay of the invention for agonists or antagonists, a eellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokine-alpha and/or Neutrokine-alphaSV such as a molecule of a signaling or regulatory pathway modulated by Neutrokine-alpha and/or Neutrokine-alphaSV. The preparation is incubated with labeled Neutrokine-alpha and/or Neutrokine-alphaSV in the absence or the presence of a candidate molecule whieh may be a Neutrokine-alpha and/or Neutrokine-alphaSV agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of Neutrokine-alpha on binding the Neutrokine-alpha and/or Neutrokine-alphaSV binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to Neutrokine-alpha and/or Neutrokine-alphaSV are agonists.

[0857] Neutrokine-alpha- and/or Neutrokine-alphaSV- like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a seeond messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of Neutrokine-alpha and/or Neutrokine-alphaSV or molecules that elicit the same effects as Neutrokine-alpha and/or Neutrokine-alphaSV. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

[0858] Another example of an assay for Neutrokine-alpha and/or Neutrokine-alphaSV antagonists is a competitive assay that combines Neutrokine-alpha and/or Neutrokine-alphaSV and a potential antagonist with membrane-bound receptor molecules or recombinant Neutrokine-alpha and/or Neutrokine-alphaSV receptor molecules under appropriate conditions for a competitive inhibition assay. Neutrokine-alpha and/or

Neutrokine-alphaSV can be labeled, such as by radioactivity, such that the number of Neutrokine-alpha and/or Neutrokine-alphaSV molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

[0859] Potential antagonists include small organic molecules, peptides, polypeptides (e.g., IL-13), and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing Neutrokine-alpha and/or Neutrokine-alphaSV induced activities, thereby preventing the action of Neutrokine-alpha and/or Neutrokine-alphaSV by excluding Neutrokine-alpha and/or Neutrokine-alphaSV from binding.

[0860] Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the extracellular domain of the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of Neutrokine-alpha and/or Neutrokine-alphaSV. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may

be expressed in vivo to inhibit production of Neutrokine-alpha and/or Neutrokine-alphaSV.

[0861] In one embodiment, the Neutrokine-alpha and/or Neutrokine-alphaSV antisense nucleic aeid of the invention is produced intracellularly by transcription from an exogenous sequenee. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Sueh a veetor would contain a sequence encoding the Neutrokine-alpha and/or Neutrokine-alphaSV antisense nueleic aeid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produee the desired antisense RNA. Sueh vectors ean be construeted by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others know in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding Neutrokine-alpha and/or Neutrokine-alphaSV, or fragments thereof, can be by any promoter known in the art to aet in vertebrate, preferably human cells. Such promoters ean be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proe. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

[0862] The antisense nueleic aeids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Neutrokine-alpha and/or Neutrokine-alphaSV gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded Neutrokine-alpha and/or Neutrokine-alphaSV antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatehes with a Neutrokine-alpha and/or Neutrokine-alphaSV RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable

degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0863] Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most effieiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of Neutrokine-alpha and Neutrokine-alphaSV shown in Figures 1A-B and 5A-B, respectively, could be used in an antisense approach to inhibit translation of endogenous Neutrokine-alpha and/or Neutrokine-alphaSV mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of Neutrokine-alpha and/or Neutrokine-alphaSV mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0864] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., Proc. Natl. Acad. Sci. 84:648-652 (1987); PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publieation No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to

another molecule, e.g., a peptide, hybridization triggered eross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0865] The antisense oligonueleotide may comprise at least one modified base moiety which is seleeted from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-aeetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic aeid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0866] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0867] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0868] In yet another embodiment, the antisense oligonucleotide is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms speeific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to eaeh other (Gautier et al., Nucl. Acids Res. 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nuel. Acids Res. 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1997)).

[0869] Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially

available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451 (1988)), etc.

[0870] While antisense nucleotides complementary to the Neutrokine-alpha and/or Neutrokine-alphaSV coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

[0871] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy Neutrokine-alpha and/or Neutrokine-alphaSV mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of Neutrokine-alpha and Neutrokine-alphaSV (Figures 1A-B and 5A-B, respectively). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Neutrokine-alpha and/or Neutrokine-alphaSV mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0872] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express Neutrokine-alpha and/or Neutrokine-alphaSV *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous Neutrokine-alpha and/or Neutrokine-alphaSV messages and inhibit

translation. Since ribozymes unlikc antisense molecules, are catalytic, a lower intracellular concentration is requircd for cfficiency.

[0873] Endogcnous gcne cxpression can also be reduced by inactivating or "knocking out" thc Ncutrokinc-alpha and/or Neutrokine-alphaSV gene and/or its promoter using targcted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capccchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrclated DNA sequcnce) flanked by DNA homologous to the endogenous polynucleotidc sequence (either thc coding regions or regulatory regions of the gene) can be used, with or without a selcctablc marker and/or a negative selectable markcr, to transfect cells that express polypeptides of thc invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0874] In other embodiments, antagonists according to the present invention include soluble forms of Neutrokine-alpha and/or Neutrokine-alphaSV (e.g., fragments of Neutrokine-alpha shown in Figures 1A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokine-alpha and/or Ncutrokine-alphaSV and fragments of Neutrokine-alphaSV shown in Figures 5A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokine-alpha and/or Neutrokine-alphaSV). Such soluble forms of the Neutrokine-alpha and/or Neutrokine-alphaSV, which may bc naturally occurring or synthetic, antagonize Neutrokine-alpha and/or Neutrokine-alphaSV mediated signaling by competing with native Neutrokine-alpha and/or Neutrokine-alphaSV for binding to

Neutrokine-alpha and/or Neutrokine-alphaSV receptors (e.g., DR5 (See, International Publication No. WO 98/41629), TR10 (See, International Publieation No. WO 98/54202), 312C2 (See, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (See, U.S. Application Serial No. 09/176,200)), and/or by forming a multimer that may or may not be capable of binding the receptor, but which is incapable of inducing signal transduction. Preferably, these antagonists inhibit Neutrokine-alpha and/or Neutrokine-alphaSV mediated stimulation of lymphocyte (e.g., B-cell) proliferation, differentiation, and/or activation. Antagonists of the present invention also inelude antibodies specific for TNF-family ligands (e.g., CD30) and Neutrokine-alpha-Fe and/or Neutrokine-alphaSV-Fc fusion proteins.

[0875] By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing and/or blocking the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), FasL, CD40L, (TNF-gamma (International Publieation No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publieation No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), neutrokine-alpha (International Publication No. WO 98/18921), CD27L, CD30L, 4-lBBL, OX40L, CD27, CD30, 4-1BB, OX40, and nerve growth factor (NGF). In preferred embodiments, the Neutrokine-alpha and/or Neutrokine-alphaSV TNF-family ligands of the invention are DR5 (See, International Publication No. WO 98/41629), TR10 (See, International Publication No. WO 98/54202), 312C2 (See, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (See, U.S. Application Serial No. 09/176,200).

[0876] Antagonists of the present invention also inelude antibodies specific for TNF-family receptors or the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention. Antibodies according to the present invention may be prepared by any of a variety of standard methods using Neutrokine-alpha and/or Neutrokine-alphaSV immunogens of the present invention. As indicated, such Neutrokine-alpha and/or Neutrokine-alphaSV immunogens include the complete Neutrokine-alpha and Neutrokine-alphaSV polypeptides depicted in Figures 1A-B (SEQ ID NO:2) and Figures

5A-B (SEQ ID NO:19), respectively, (which may or may not include the leader sequence) and Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide fragments comprising, for example, the ligand binding domain, TNF-conserved domain, extracellular domain, transmembrane domain, and/or intraeellular domain, or any combination thereof.

[0877] Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992)); Tartaglia et al., *Cell* 73:213-216 (1993)), and PCT Application WO 94/09137 and are preferably specific to (i.e., bind uniquely to polypeptides of the invention having the amino acid sequence of SEQ ID NO:2. The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to inelude intact moleeules as well as fragments thereof (such as, for example, Fab and F(ab') fragments) which are capable of binding an antigen. Fab, Fab' and F(ab') fragments lack the Fe fragment intact antibody, clear more rapidly from the eireulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.*, 24:316-325 (1983)).

[0878] In a preferred method, antibodies aceording to the present invention are mAbs. Sueh mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow et al., *Antibodies*: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon et al., eds.), Elsevier, Amsterdam (1984)).

[0879] Proteins and other compounds which bind the Neutrokine-alpha and/or Neutrokine-alphaSV domains are also candidate agonists and antagonists according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two- hybrid system has been described by Roger Brent and his colleagues (Gyuris, *Cell* 75:791-803 (1993); Zervos et al., *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to the ligand binding domain, extracellular, intracellular, transmembrane, and death

domain of the Neutrokine-alpha and/or Neutrokine-alphaSV. Sueh compounds are good candidate agonists and antagonists of the present invention.

[0880] For example, using the two-hybrid assay described above, the extraeellular or intracellular domain of the Neutrokine-alpha and/or Neutrokine-alphaSV receptor, or a portion thereof, may be used to identify eellular proteins whieh interact with Neutrokine-alpha and/or Neutrokine-alphaSV the receptor *in vivo*. Such an assay may also be used to identify ligands with potential agonistic or antagonistic activity of Neutrokine-alpha and/or Neutrokine-alphaSV receptor function. This screening assay has previously been used to identify protein which interact with the eytoplasmie domain of the murine TNF-RII and led to the identification of two receptor associated proteins. Rothe et al., *Cell* 78:681 (1994). Such proteins and amino acid sequences which bind to the cytoplasmic domain of the Neutrokine-alpha and/or Neutrokine-alphaSV receptors are good candidate agonist and antagonist of the present invention.

[0881] Other screening teehniques include the use of cells which express the polypeptide of the present invention (for example, transfected CHO eells) in a system which measures extracellular pH ehanges caused by reeeptor activation, for example, as described in *Science*, 246:181-296 (1989). In another example, potential agonists or antagonists may be contaeted with a cell whieh expresses the polypeptide of the present invention and a second messenger response, e.g., signal transduction may be measured to determine whether the potential antagonist or agonist is effective.

[0882] Agonists according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth faetor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science* 267:1457-1458 (1995)).

[0883] Preferred agonists are fragments of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention which stimulate lymphocyte (e.g., B cell) proliferation, differentiation and/or activation. Further preferred agonists include polyclonal and monoclonal antibodies raised against the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention, or a fragment thereof. Such agonist

antibodies raised against a TNF-family receptor are disclosed in Tartaglia et al., *Proc. Natl. Acad. Sci. USA* 88:9292-9296 (1991); and Tartaglia et al., *J. Biol. Chem.* 267:4304-4307(1992). See, also, PCT Application WO 94/09137.

[0884] In an additional embodiment, immunoregulatory molecules such as, for example, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha, may be used as agonists of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention which stimulate lymphoeyte (e.g., B cell) proliferation, differentiation and/or activation. In a specific embodiment, IL4 and/or IL10 are used to enhance the Neutrokine-alpha- and/or Neutrokine-alphaSV-mediated proliferation of B eells.

[0885] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such eells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The eells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the eoding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral veetors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, eleetroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

[0886] Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and

Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is ineorporated by reference herein in its entirety).

[0887] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced eells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

[0888] In yet another embodiment of the invention, the aetivity of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide can be reduced using a "dominant negative." To this end, constructs which eneode defective Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide, such as, for example, mutants laeking all or a portion of the TNF-conserved domain, can be used in gene therapy approaehes to diminish the activity of Neutrokine-alpha and/or Neutrokine-alphaSV on appropriate target cells. For example, nucleotide sequences that direct host eell expression of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in which all or a portion of the TNF-conserved domain is altered or missing can be introduced into monocytic cells or other cells or tissues (either by in vivo or ex vivo gene therapy methods described herein or otherwise known in the art). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous Neutrokine-alpha and/or Neutrokine-alphaSV gene in monocytes. The engineered cells will express non-functional Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides (i.e., a ligand (e.g., multimer) that may be capable of binding, but which is incapable of inducing signal transduction).

Chromosome Assays

[0889] The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few ehromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes

according to the present invention is an important first step in correlating those sequences with genes associated with disease.

[0890] In certain preferred embodiments in this regard, the cDNA and/or polynueleotides herein disclosed is used to elone genomic DNA of a Neutrokine-alpha and/or Neutrokine-alphaSV gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

[0891] In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This teehnique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al., Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

[0892] Once a sequence has been mapped to a preeise chromosomal location, the physical position of the sequenee on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0893] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

[0894] With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be

one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

[0895] Utilizing the techniques described above, the chromosomal location of Neutrokine-alpha and Neutrokine-alphaSV was determined with high confidence using a combination of somatic cell hybrids and radiation hybrids to chromosome position 13q34.

Examples

[0896] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting. Many of the following examples are set forth referring specifically to Neutrokine-alpha polynucleotides and polypeptides of the invention. Each example may also be practiced to generate and/or examine Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention. One of ordinary skill in the art would easily be able to direct the following examples to Neutrokine-alphaSV.

Example 1a: Expression and Purification of "His-tagged" Neutrokine-alpha in E. coli

[0897] The bacterial expression vector pQE9 (pD10) is used for bacterial expression in this example. (QIAGEN, Inc., *supra*). pQE9 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra,* and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide.

[0898] The DNA sequence encoding the desired portion of the Neutrokine-alpha protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to

facilitate cloning in the pQE9 vector are added to the 5' and 3' primer sequences, respectively.

[0899] For cloning the extracellular domain of the protein, the 5' primer has the sequence 5'-GTG GGA TCC AGC CTC CGG GCA GAG CTG-3' (SEQ ID NO:10) containing the underlined *Bam* HI restriction site followed by 18 nucleotides of the amino terminal coding sequence of the extracellular domain of the sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete Neutrokine a protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:11) containing the underlined *Hind* III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence of the DNA sequence in Figures 1A and 1B.

[0900] The amplified DNA fragment and the vector pQE9 are digested with *Bam* HI and *Hind* III and the digested DNAs are then ligated together. Insertion of the DNA into the restricted pQE9 vector places the protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

[0901] The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing. Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to

1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-beta-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0902] The cells are then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the is loaded on to a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded on to the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide is eluted with 6 M guanidine-HCl, pH 5.

[0903] The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

Example 1b: Expression and Purification of Neutrokine-alpha in E. coli

[0904] The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single

restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

[0905] The DNA sequence encoding the desired portion of the protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers whieh anneal to the amino terminal sequences of the desired portion of the protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

[0906] For cloning the extracellular domain of the protein, the 5' primer has the sequence 5'-GTG TCA TGA GCC TCC GGG CAG AGC TG-3' (SEQ ID NO:12) containing the underlined *Bsp* HI restriction site followed by 17 nucleotides of the amino terminal coding sequence of the extracellular domain of the sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the eomplete protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:13) containing the underlined *Hin*d III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence in the DNA sequenee in Figures 1A and 1B.

[0907] The amplified DNA fragments and the vector pQE60 are digested with *Bsp* HI and *Hin*d III and the digested DNAs are then ligated together. Insertion of the DNA into the restricted pQE60 vector places the protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The assoeiated stop codon prevents translation of the six histidine codons downstream of the insertion point.

[0908] The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

E. coli strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

[0909] One of ordinary skill in the art recognizes that any of a number of bacterial expression vectors may be useful in place of pQE9 and pQE60 in the expression protocols presented in this example. For example, the novel pHE4 series of bacterial expression vectors, in particular, the pHE4-5 vector may be used for bacterial expression in this example (ATCC Accession No. 209311; and variations thereof). The plasmid DNA designated pHE4-5/MPIFD23 in ATCC Deposit No. 209311 is vector plasmid DNA which contains an insert which encodes another ORF. The construct was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 30, 1997. Using the *Nde* I and *Asp* 718 restriction sites flanking the irrelevant MPIF ORF insert, one of ordinary skill in the art could easily use current molecular biological techniques to replace the irrelevant ORF in the pHE4-5 vector with the Neutrokine-alpha ORF of the present invention.

[0910] The pHE4-5 bacterial expression vector includes a neomycin phosphotransferase gene for selection, an *E. coli* origin of replication, a T5 phage promoter sequence, two *lac* operator sequences, a Shine-Delgarno sequence, and the lactose operon repressor gene (*lac*Iq). These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide. The promoter and operator sequences of the pHE4-5 vector were made synthetically. Synthetic production of nucleic acid sequences is well known in the art (CLONETECH 95/96 Catalog, pages 215-216, CLONETECH, 1020 East Meadow Circle, Palo Alto, CA 94303).

[0911] Clones containing the desired Neutrokine-alpha constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution

of approximately 1:25 to 1:250. The eells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. isopropyl-beta-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0912] The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the Neutrokine a is dialyzed against 50 mM Na-acetate buffer pH 6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure protein. The purified protein is stored at 4°C or frozen at -80° C.

[0913] In certain embodiments, it is preferred to generate expression constructs as detailed in this Example to mutate one or more of the three cysteine residues in the Neutrokine-alpha polypeptide sequence. The cysteine residues in the Neutrokine-alpha polypeptide sequence are located at positions 147, 232, and 245 as shown in SEQ ID NO:2 and at positions 213 and 226 of the Neutrokine-alpha polypeptide sequence as shown in SEQ ID NO:19 (there is no eysteine in the Neutrokine-alphaSV polypeptide sequence which corresponds to Cys-147 in the Neutrokine-alpha polypeptide sequence because amino acid residues 143-160 of the Neutrokine-alpha polypeptide sequence are not present in the Neutrokine-alphaSV polypeptide sequenee).

Example 2: Cloning, Expression, and Purification of Neutrokine-alpha Protein in a Baculovirus Expression System

[0914] In this illustrative example, the plasmid shuttle vector pA2GP is used to insert the cloned DNA encoding the extracellular domain of the protein, lacking its naturally associated intracellular and transmembrane sequences, into a baculovirus to express the extracellular domain of the Neutrokine-alpha protein, using a baculovirus leader and standard methods as described in Summers et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station

Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein and convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

[0915] Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology 170*:31-39 (1989).

[0916] The cDNA sequence encoding an N-terminally deleted form of the extracellular domain of the Neutrokine-alpha protein in the deposited clone, lacking the AUG initiation codon, the naturally associated intracellular and transmembrane domain sequences, and amino acids Gln-73 through Leu-79 shown in Figures 1A and 1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-GTG GGA TCC CCG GGC AGA GCT GCA GGG C-3' (SEQ ID NO:14) containing the underlined *Bam* HI restriction enzyme site followed by 18 nucleotides of the sequence of the extracellular domain of the Neutrokine-alpha protein shown in Figures 1A and 1B, beginning with the indicated N-terminus of the extracellular domain of the protein. The 3' primer has the sequence 5'-GTG GGA TCC TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:15) containing the underlined *Bam* HI restriction site followed by two stop codons and 18 nucleotides complementary to the 3' coding sequence in Figures 1A and 1B.

[0917] In certain other embodiments, constructs designed to express the entire predicted extracellular domain of the Neutrokine-alpha (i.e., amino acid residues Gln-73 through Leu-285) are preferred. One of skill in the art would be able to use the

polynucleotide and polypeptide sequences provided as SEQ ID NO:1 and SEQ ID NO:2, respectively, to design polynucleotide primers to generate such a clone.

[0918] In a further preferred embodiment, a pA2GP expression construct encodes amino acid residues Leu-112 through Leu-285 of the Neutrokine-alpha polypeptide sequence shown as SEQ ID NO:2.

[0919] In another preferred embodiment, a pA2GP expression construct encodes amino acid residues Ser-78 through Leu-285 of the Neutrokine-alpha polypeptide sequence shown as SEQ ID NO:2.

[0920] The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with *Bam* HI and again is purified on a 1% agarose gel. This fragment is designated herein F1.

[0921] The plasmid is digested with the restriction enzymes *Bam* HI and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

[0922] Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human gene by digesting DNA from individual colonies using *Bam* HI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2GP-Neutrokine-alpha.

[0923] Five micrograms of the plasmid pA2GP-Neutrokine-alpha is co-transfected with 1.0 microgram of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA 84*: 7413-7417 (1987). One μg of BaculoGold™ virus DNA and 5 micrograms of the plasmid pA2GP Neutrokine-alpha are mixed in a sterile well of a microtiter plate containing 50 microliters of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards,

10 microliters Lipofectin plus 90 microliters Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27°C for four days.

[0924] After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Rockville, Maryland) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 microliters of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-Neutrokine-alpha.

[0925] To verify the expression of the Neutrokine-alpha gene Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-Neutrokine-alpha at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 microcuries of ^{35}S-methionine and 5 microcuries ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

[0926] Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the extracellular domain of the protein and thus the cleavage point and length of the secretory signal peptide.

[0927] In a specific experimental example, recombinant Neutrokine-alpha was purified from baculovirus infected Sf9 cell supernatants as follows. The insect cells were grown in EXCEL401 medium (JRH Scientifie) with 1 % (v/v) fetal bovine serum. At 92 hours post-infcetion, the harvested supernatant was clarified by centrifugation at 18,000 x *g* followed by 0.45 m depth filtration. A de-lipid filtration step might be also used to remove the lipid contaminants and in turn to improve initial capturing of the Neutrokine-alpha protein.

[0928] The supernatant was loaded on to a set of Poros HS-50/HQ-50 in tandem mode. As alternatives, Toyopearl QAE, Toyopearl Super Q (Tosohass), Q-Sepharose (Pharmacia) and equivalent resins might be used. This step is used as a negative purification step to remove strong anion binding contaminants. The HS/HQ flow through material was adjusted to pH 7.5 with 1 M Tris-HCl pH 8, diluted with equal volume of 50 mM Tris-HCl pH 8, and loaded onto a poros PI-20 or PI-50 column. The PI column was washed first with 4 column volumes of 75 mM sodium chloride in 50 mM Tris-HCl at pH 7.5, then eluted using 3 to 5 column volumes of a stepwise gradient of 300 mM, 750 mM, 1500 mM sodium chloride in 50 mM Tris-HCl pH 7.5. Neutrokine-alpha protein appears as a 17 KD band on reduced SDS-PAGE and is present in the 0.75 M to 1.5M Sodium chloride fractions.

[0929] The PI fraction was further purified through a Sephacryl S100 HR (Pharmacia) size exclusion column equilibrated with 0.15 M sodium chloride, 50 mM sodium aeetate at pH 6. The S200 fractions were mixed with sodium chloride to a final concentration of 3 M and loaded onto a Toyopearl Hexyl 650C (Tosohass) column. The Hexyl column was eluted with a linear gradient from 3 M to 0.05 M sodium chloride in 50 mM Sodium acetate pH 6 in 5 to 15 column volumes. The sodium chloride gradient can also be replaced by ammonium sulfate gradient of 1M to 0 M in 50 mM sodium acetate pH 6 in the Hexyl chromatographic step. Fractions containing purified Neutrokine-alpha as analyzed through SDS-PAGE were combined and dialyzed against a buffer containing 150 mM Sodium chloride, 50 mM Sodium acetate, pH 6.

[0930] The final purified Neutrokine-alpha protein expressed in a baculovirus system as explained herein has an N-terminus sequence whieh begins with amino acid residue Ala-134 of SEQ ID NO:2. RP-HPLC analysis shows a single peak of greater than 95% purity. Endotoxin level was below the detection limit in LAL assay.

[0931] In another example, recombinant Neutrokine-alpha was purified from baculovirus infected Sf9 cell supernatants containing 0.25% bovine serum as follows.

[0932] The Sf9 supernatant was harvested by centrifugation at 18,000 x *g*. The supernatant was then treated with 10 mM calcium chloride in slightly alkaline conditions for 10-15 minutes followed by centrifugation and then 0.22 micrometer depth filtration. The resulting Sf-9 cell supernatant was then diluted 2-fold and loaded on to a Poros PI-50 column (available from PE Biosystems). The column was equilibrated with 50 mM Tris (pH=7.4). The PI-50 column was washed with 1 CV of 50 mM Tris (pH=7.4) and then eluted with 1.5 M NaCl in 50 mM NaOAc (pH=6) over 3 CV. The PI fraction was loaded on to a Sephacryl S200 column equilibrated with 50 mM NaOAc (pH=6), 125 mM NaCl. The S200 fraction was mixed with salts to final concentrations of 0.7 M ammonium sulfate and 0.6 M NaCl and loaded on to a Toyopearl Hexyl 650C column (available from Toso Haas) that had been equilibrated in a buffer containing 0.6 M NaCl, 0.7 M ammonium sulfate in 50 mM NaOAc (pH=6). The column was then washed with 2 CV of the same buffer. Recombinant Neutrokine-alpha was then eluted stepwise with 3 CV of 50 mM NaOAc (pH=6) followed by 2 CV of 20% ethanol wash. The recombinant Neutrokine-alpha protein was then eluted at the end of the ammonium sulfate (0.3 to 0 M salt) gradient. The appropriate fractions were pooled and dialyzed against a buffer containing 50 mM NaOAc (pH=6), and then passed through a Poros 50 HQ column. The HQ flow-through was diluted to 4 ms and loaded on to a Toyopearl DEAD 650M column and then eluted with 25 mM NaCitrate, 125 mM NaCl.

[0933] In another example, recombinant Neutrokine-alpha was expressed and purified using a baculoviral vector system in Sf+ insect cells.

[0934] First, a polynueleotide encoding amino acid residues Ser-78 through Leu-285 of the Neutrokine-alpha polypeptide sequenee shown in Figures 1A and 1B (which is exactly identical to amino acid residues Ser-78 through Leu-285 of the Neutrokine-alpha polypeptide sequenee shown as SEQ ID NO:2) was subeloned into the baculovirus transfer eonstruet PSC to generate a baculovirus expression plasmid. The pA2GP transfer vector, derived from pVL941, contains the gp67 signal peptide, a modified multiple cloning site, and the *lac Z* gene cloned downstream of the *Drosophila* heat-shock promoter for selection of blue plaques. Using the sequence of Neutrokine-alpha (SEQ ID NO:2) and the sequence of the pA2GP vector, a cloning strategy was designed for

seamlessly fusing the PSC signal peptide coding sequence to the Neutrokine-alpha coding sequence at Ala-134 (SEQ ID NO:2 and Figures 1A and 1B) and inserting it into a PSC baculovirus transfer plasmid. The strategy involved the use of a two-stage polymerase chain reaction (PCR) procedure. First, primers were designed for amplifying the Neutrokine-alpha sequenees. The 5' primer consisted of the sequence encoding Ala-134 and following residues (5'-GGT CGC CGT TTC TAA CGC GGC CGT TCA GGG TCC AGA AG-3'; SEQ ID NO:31), preceded by the sequence encoding the PSC signal peptide C-terminus. The 3' primer (5'-CTG GTT CGG CCC AAG GTA CCA AGC TTG TAC CTT AGA TCT TTT CTA GAT C-3'; SEQ ID NO:32) consisted of the reverse complement of the pA2GP veetor sequence immediately downstream from the Neutrokine-alpha coding sequence, preceded by a *Kpn* I restriction endonuclease site and a spacer sequence (for increased cutting efficiency by *Kpn* I). PCR was performed with the pA2GP containing Neutrokine-alpha plasmid template and primers O-1887 and O-1888, and the resulting PCR product was purified using standard techniques.

[0935] An additional PCR reaction was performed using the PSC baculovirus transfer plasmid pMGS12 as a template. The pMGS12 plasmid consists of the AcNPV EcoRI "I" fragment inserted into pUC8, with the polyhedrin coding sequences after the ATG start codon replaced with the PSC signal peptide and a polylinker site. The PCR reaction used pMGS12 as a template, a 5' primer (5'-CTG GTA GTT CTT CGG AGT GTG-3'; SEQ ID NO:33) which annealed in AcNPV ORF603 upstream of the unique *Ngo*M IV and *Eco*R V sites, and a 3' primer (5'-CGC GTT AGA AAC GGC GAC C-3'; SEQ ID NO:34) which annealed to the 3' end of the sequence encoding the PSC signal peptide.

[0936] To generate a PCR product in which the PSC signal peptide was seamlessly fused to the Ala-134 of the Neutrokine-alpha coding sequence, the PCR product was eombined with the PSC signal peptide-polyhedrin upstream region PCR product and subjected to an additional round of PCR. Because the 3' end of the PSC signal peptide PCR product (pMGS12 / O-959 / O-1044) overlapped the 5' end of the Neutrokinc-alpha PCR product prepared with primers O-1887 / O-1888, the two PCR produets were combined and overlap-extended by PCR using primers O-959 and O-1888.

[0937] The resulting overlap-extended PCR product eontaining the PSC signal peptide fused to the Neutrokine-alpha sequence subsequently was inserted into baculovirus transfer plasmid pMGS12. The PCR product was digested with *Ngo*M IV and *Kpn* I, and

the fragment was purified and ligated into *Ngo*M IV-*Kpn* I-cut pMGS12. After transformation of competent *E. coli* DH5alpha cells with the ligation mix, colonies were picked and plasmid DNA mini-preps were prepared. Several positive clones from each ligation were identified by restriction digestion analysis of the plasmid DNA, and three clones (pAcC9669, pAcC9671, and pAcC9672) were selected for large scale plasmid purification. The resulting plasmid DNA was subjected to DNA sequence analysis to confirm and sequence the Neutrokine-alpha insert.

[0938] The following steps deseribe the recovery and purification process of recombinant Neutrokine-alpha from Sf+ insect cells. Unless stated otherwise, the process is eondueted at 2-8°C.

Recovery

Step 1. $CaCl_2$ Treatment

[0939] Sf+ cell supernatant was harvested by centrifugation at 8,000 x *g*. Recovery buffer-1 (1M $CaCl_2$) was added to the supernatant so that the final concentration of $CaCl_2$ was 10 mM. (In a further preferred embodiment, 1M $ZnCl_2$ is used in place of 1M $CaCl_2$.) The pH of the solution was adjusted to 7.7 ± with Recovery buffer-2 (1M Tris pH 8 (± 0.2)). The solution was incubated for 15 minutes and then centrifuged at 8,000 x *g*.

Purification

Step 1. Chromatography on Poros PI-50 Column

[0940] Sf+ cell supernatant was loaded on to a Poros PI-50 column (PE Biosystem). The column was equilibrated in PI-1 buffer (50 mM Tris, 50 mM NaCl, pH 7.4 (± 0.2)). The PI-50 column was washed with 1-2 CV of PI-1 buffer and then eluted with PI-2 buffer (50 mM Na Citrate pH 6 (± 0.2)) over 3 CV linear gradient. The elution was monitored by ultraviolet (UV) absorbance at 280 nm. Fractions were eolleeted across the eluate peak and analyzed by SDS page. Appropriate fraetions were pooled.

Step 2. Chromatography on Toyopearl Hexyl 650C Column

[0941] The PI pool was mixed with salts to final coneentrations of 0.7M $(NH_4)_2SO_4$ and loaded on to a Toyopearl Hexyl 650C (Toso Haas) column equilibrated in HIC-1 buffer (50 mM NaOAc, 0.6M NaCl, 0.7M $(NH_4)_2SO_4$ pH 6 (± 0.2)). The column was then

washed with 2 CV of HIC-1 buffer. Subsequently, recombinant Neutrokine-alpha was then eluted stepwise with 3-5 CV of HIC-2 buffer (50mM NaOAc pH 6.0 (± 0.2)) followed by a 2 CV 20% ethanol wash. The elution was monitored by UV absorbance at 280 nm and conductivity. Fractions were collected across the eluate peak and analyzed by SDS-PAGE. The appropriate fractions were then pooled.

Step 3. Chromatography on SP sepharose FF

[0942] The Hexyl fraetion was dialyzed and ajusted to pH 4.5 with SP-1 buffer (50 mM sodium acetate pH 4.5 (± 0.2)), diluted to 4 ms and loaded through a SP sepharose (cation exchanger, Pharmacia) column equilibrated with SP-1 buffer (50 mM sodium acetate pH 4.5 (± 0.2)). Reecombinant Neutrokine-alpha protein was then eluted from the SP column with SP-2 buffer (50 mM sodium acetate pH 5.5 (± 0.2)) at pH 5.5. The elution was then monitored by ultraviolet (UV) absorbance at 280 nm. Fractions were collected across the eluate peak and analyzed by SDS page. Appropriate fractions were pooled.

Step 4. Dialysis of Recombinant Neutrokine-alpha

[0943] The SP fractions were placed into a 6-8 kd cutoff membrane device and then dialyzed or diafiltered into Dialysis Buffer (10 mM sodium citrate, 140 mM sodium chloride pH 6 (± 0.2)) overnight.

Step 5. Filtration and Fill

[0944] The protein concentration of the recombinant Neutrokine-alpha solution from Step 6 was determined by bicinchoninic acid (BCA) protein assay. Recombinant Neutrokine-alpha formulation was adjusted to the final protein concentration with the appropriate buffer and filtered under controlled conditions. The filtrate (bulk substance) was stored in suitable sterilized containers below -20°C.

[0945] In a specific embodiment, Neutrokine-alpha protein of the invention produced as described infra was adjusted to a final protein concentration of 1 to 5 mg/ml and buffered in 10 mM sodium citrate, 140 mM sodium chloride, pH = 6.0 ± (0.4) and stored at or below -20°C in Type 1 glass vials.

[0946] During chromatography runs, the processes are monitered by UV absorbance at 280 nm. When applicable, in-process chromatography intermediates are tested for conductivity, pH, and monitored by SDS and/or RP-HPLC.

[0947] Columns and purification equipment are cleaned and sanitized with 0.2 or 0.5 M NaOH followed by deionized water and then 0.1 or 0.5 M acetic acid. The column and purification equipment are rinsed with deionized water and, if necessary, stored in the appropriate storage solution. Prior to use, the equipment is equilibrated with appropriate buffers (as described herein or as is well known in the art).

[0948] In a further preferred embodiment, 1M $ZnCl_2$ is used in place of 1M $CaCl_2$ in Step 1 of the Recovery section described above. Also, in this embodiment, a combination of $ZnCl_2$ and $CaCl_2$ may be used. Many combinations of 0.1 M $ZnCl_2$ and 0.9 M $CaCl_2$, may be used in the Recovery process of recombinant Neutrokine-alpha protein such as, for example, but not limited to, a combination of 0.1 M $ZnCl_2$ and 0.9 M $CaCl_2$, 0.2 M $ZnCl_2$ and 0.8 M $CaCl_2$, 0.3 M $ZnCl_2$ and 0.7 M $CaCl_2$, 0.4 M $ZnCl_2$ and 0.6 M $CaCl_2$, 0.5 M $ZnCl_2$ and 0.5 M $CaCl_2$, 0.6 M $ZnCl_2$ and 0.4 M $CaCl_2$, 0.7 M $ZnCl_2$ and 0.3 M $CaCl_2$, 0.8 M $ZnCl_2$ and 0.2 M $CaCl_2$, 0.9 M $ZnCl_2$ and 0.1 M $CaCl_2$, and others. However, the presenee of EDTA will inhibit the recovery process. Moreover, the presence of $ZnCl_2$ and/or $CaCl_2$ in Recovery Buffer-1 will induce the formation of larger amounts of higher molecular weight (or molecular mass) Neutrokine-alpha multimers.

Example 3: Cloning and Expression of Neutrokine-alpha in Mammalian Cells

[0949] A typical mammalian expression vector contains the promoter element, whieh mediates the initiation of transcription of mRNA, the protein eoding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in praeticing the present invention include, for example, vectors sueh as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVeat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109).

Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells, Chinese hamster ovary (CHO) cells CHO-K1, NSO and HEK 293 cells.

[0950] Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

[0951] The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J. 227*:277-279 (1991); Bebbington et al., *Bio/Technology 10*:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

[0952] The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

[0953] The expression plasmid, pNeutrokine-alpha-HA, is made by cloning a portion of the deposited cDNA encoding the extracellular domain of the protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.). To produce a soluble, secreted form of the polypeptide, the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene.

[0954] The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an

SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson et al., *Cell 37*: 767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

[0955] A DNA fragment encoding the extracellular domain of the Neutrokine-alpha polypeptide is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The Neutrokine-alpha cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of Neutrokine-alpha in *E. coli*. Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined *Bam* HI site, a Kozak sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neutrokine-alpha protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam* HI restriction site and 18 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

[0956] The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with *Bam* HI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is

isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the Neutrokine-alpha extracellular domain.

[0957] For expression of recombinant Neutrokine-alpha, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of Neutrokine-alpha by the vector.

[0958] Expression of the Neutrokine-alpha-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow et al., *Antibodies: A Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

[0959] The vector pC4 is used for the expression of Neutrokine-alpha protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). To produee a soluble, secreted form of the Neutrokine-alpha polypeptide, the portion of the deposited eDNA encoding the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene. The vector plasmid contains the mouse DHFR gene under eontrol of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the eells in a seleetive medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem. 253*:1357-1370, Hamlin, J.

L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing coneentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

[0960] Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, et al., *Molecular and Cellular Biology*, March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell 41*:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human beta-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the Neutrokine-alpha in a regulated way in mammalian eells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

[0961] The plasmid pC4 is digested with the restriction enzymes *Bam* HI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

[0962] The DNA sequence encoding the extracellular domain of the Neutrokine-alpha protein is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer, containing the underlined *Bam* HI site, a Kozak

sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neutrokine-alpha protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam* HI and 18 of nucleotides complementary to the 3' coding sequenee immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

[0963] The amplified fragment is digested with the endonuclease Bam HI and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

[0964] Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the eells are trypsinized and seeded in hybridoma eloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 μM, 20 μM). The same proeedure is repeated until clones are obtained whieh grow at a coneentration of 100-200 μM. Expression of the desired gene produet is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

[0965] At least six Neutrokine-alpha expression eonstructs have been generated by the inventors herein to faeilitate the production of Neutrokine-alpha and/or

Neutrokine-alphaSV polypeptides of several sizes and in several systems. The expression constructs are as follows: (1) pNa.A71-L285 (expresses amino acid residues Ala-71 through Leu-285), (2) pNa.A81-L285 (expresses amino acid residues Ala-81 through Leu-285), (3) pNa.L112-L285 (expresses amino acid residues Leu-112 through Leu-285), (4) pNa.A134-L285 (expresses amino acid residues Ala-134 through Leu-285), (5) pNa.L147-L285 (expresses amino acid residues Leu-147 through Leu-285), and (6) pNa.G161-L285 (expresses amino acid residues Gly-161 through Leu-285).

[0966] In preferred embodiments, the expression constructs are used to express various Neutrokine-alpha muteins from bacterial, baculoviral, and mammalian systems.

[0967] In certain additional preferred embodiments, the constructs express a Neutrokine-alpha polypeptide fragment fused at the N- and/or C-terminus to a heterologous polypeptide, e.g., the signal peptide from human IL-6, the signal peptide from CK-beta8 (amino acids -21 to -1 of the CK-beta8 sequence disclosed in published PCT application PCT/US95/09058), or the human IgG Fc region. Other sequences could be used which are known to those of skill in the art.

Example 4: Tissue distribution of Neutrokine-alpha mRNA expression

[0968] Northern blot analysis is carried out to examine Neutrokine-alpha gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the Neutrokine-alpha protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Scienee), aceording to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufaeturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for Neutrokine-alpha and/or Neutrokine-alpha mRNA.

[0969] Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70° C overnight, and films developed according to standard procedures.

[0970] To determine the pattern of Neutrokine-alpha and/or Neutrokine-alpha expression a panel of multiple tissue Northern blots were probed. This revealed predominant expression of single 2.6 kb mRNA in peripheral blood leukocytes, spleen, lymph node and bone marrow, and detectable expression in placenta, heart, lung, fetal liver, thymus and pancreas. Analysis of a panel of cell lines demonstrated high expression of Neutrokine-alpha and/or Neutrokine-alpha in HL60 cells, detectable expression in K562, but no expression in Raji, HeLa, or MOLT-4 cells. Overall it appears that Neutrokine-alpha and/or Neutrokine-alpha mRNA expression is enriched in the immune system.

Example 5: Gene Therapy Using Endogenous Neutrokine-alpha Gene

[0971] Another method of gene therapy aceording to the present invention involves operably assoeiating the endogenous Neutrokine-alpha sequence with a promoter via homologous recombination as described, for example, in U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publieation No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target eells, but whieh is not expressed in the cells, or is expressed at a lower level than desired. Polynueleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous Neutrokine-alpha, flanking the promoter. The targeting sequence will be suffieiently near the 5' end of Neutrokine-alpha so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences ean be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

[0972] The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together

in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

[0973] In this Example, the polynueleotide constructs are administered as naked polynucleotides via eleetroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, preeipitating agents, etc. Such methods of delivery are known in the art.

[0974] Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous Neutrokine-alpha sequenee. This results in the expression of Neutrokine-alpha in the cell. Expression may be detected by immunologieal staining, or any other method known in the art.

[0975] Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is plaeed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjeeted to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2 HPO4, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately $3X10^6$ cells/ml. Electroporation should be performed immediately following resuspension.

[0976] Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the Neutrokine-alpha locus, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two Neutrokine-alpha non-coding sequences are amplified via PCR: one Neutrokine-alpha non-coding sequence (Neutrokine-alpha fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other Neutrokine-alpha non-coding sequence (Neutrokine-alpha fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and Neutrokine-alpha fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; Neutrokine-alpha fragment 1 - XbaI; Neutrokine-alpha fragment 2 - BamHI) and ligated together. The resulting

ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

[0977] Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately 1.5.X106 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

[0978] Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37°C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

[0979] The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 6: Neutrokine-alpha, a Novel Member of the Tumor Necrosis Factor Ligand Family that Functions as a B Lymphocyte Stimulator

[0980] A 285 amino acid protein was identified in a human neutrophil/monocyte-derived cDNA library that shared significant homology within its predicted extracellular receptor-ligand binding domain to APRIL (28.7%) (Hahne, M., et al., *J.Exp.Med.* 188,1185-90 (1998)), TNF-alpha (16.2%) (Pennica, D., et al., *Nature* 312,724-729 (1984)) and LT-alpha (14.1%) (Gray, *Nature* 312,721-724 (1984)) (Figures 7A-1 and 7A-2). We have designated this cytokine Neutrokine-alpha (we have also designated this molecule as B Lymphocyte Stimulator (BLyS) based on its biological activity). Hydrophobicity analyses of the the Neutrokine-alpha protein sequence have revealed a potential transmembrane spanning domain between amino acid residues 47 and 73 which

is preceded by non-hydrophobic amino acids suggesting that Neutrokine-alpha, like other members of the TNF ligand family, is a type II membrane bound protein (Cosman, D. *Stem.Cells.* 12:440-55 (1994)). Expression of this cDNA in mammalian cells (HEK 293 and Chinese Hamster Ovary) and Sf9 inseet cells identified a 152 amino acid soluble form with an N-terminal sequence beginning with the alanine residue at amino acid 134 (arrow in Figures 7A-1 and 7A-2). Reconstruction of the mass to charge ratio defined a mass for Neutrokine-alpha of 17,038 Daltons, a value in consistent with that predicted for this 152 amino acid protein with a single disulfide bond (17037.5 Daltons).

[0981] Using human/hamster somatic cell hybrids and a radiation-hybrid mapping panel, the gene encoding Neutrokine-alpha was found linked to marker SHGC-36171 which maps to human chromosome 13q34, a region not previously associated with any other member of the TNF superfamily of genes (Cosman, D. *Stem.Cells.* 12:440-55 (1994)).

[0982] The expression profile of Neutrokine-alpha was assessed by Northern blot (Figure 7B) and flow cytometric analyses (Table V and Figures 8A, 8B and 8C). Neutrokine-alpha is encoded by a single 2.6kb mRNA found at high levels in peripheral blood leukocytes, spleen, lymph node and bone marrow. Lower expression levels were detected in placenta, heart, lung, fetal liver, thymus and pancreas. Among a panel of eell lines, Neutrokine-alpha mRNA was deteeted in HL-60 and K562, but not in Raji, HeLa, or MOLT-4 cells. These results were confirmed by flow cytometric analyses using the Neutrokine-alpha-specific mAb 2E5. As shown in Table V, Neutrokine-alpha expression is not detected on T or B lineage cells but rather restricted to cells within the myeloid origin. Further analyses of normal blood cell types demonstrated significant expression on resting monocytes that was upregulated approximately 4-fold following exposure of cells to IFN-gamma (100 U/mL) for three days (Figures 8A and 8B). A concomitant increase in Neutrokine-alpha-specific mRNA was also detected (Figure 8C). By contrast, Neutrokine-alpha was not expressed on freshly isolated peripheral blood granulocytes, T cells, B cells, or NK cells.

[0983] Purified recombinant Neutrokine-alpha ("rNeutrokine-alpha") was assessed for its ability to induce activation, proliferation, differentiation or death in numerous cell based assays involving B cells, T cells, monocytes, NK cells, hematopoietic progenitors, and a variety of cell types of endothelial and epithelial origin. Among these assays,

Neutrokine-alpha was specifically found to increase B cell proliferation in a standard co-stimulatory assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM as priming agents (Sieckmann, D.G., *et al., J.Exp.Med.* 147:814-29 (1978); Ringden, O., *et al., Scand.J.Immunol.* 6:1159-69 (1977)). As shown in Figure 9A, recombinant Neutrokine-alpha induced a dose-dependent proliferation of tonsillar B cells. This response was similar to that of rIL2 over the dose range from 0.1 to 10,000 ng/mL. Neutrokine-alpha also induces B cell proliferation when cultured with cells co-stimulated with immobilized anti-IgM (Figure 9B). A dose-dependent response is readily observed as the amount of crosslinking agent increases in the presence of a fixed concentration of either IL2 or rNeutrokine-alpha.

[0984] In an attempt to correlate the specific biological activity on B cells with receptor expression, purified Neutrokine-alpha was biotinylated. The resultant biotin-Neutrokine-alpha protein retained biological function in the standard B cell proliferation assays. Lineage-specific analyses of whole human peripheral blood cells indicated that binding of biotinylated Neutrokine-alpha was undetectable on T eells, monoeytes, NK eells and granulocytes as assessed by CD3, CD14, CD56, and CD66b respectively (Figures 10A, 10B, 10C, 10D and 10E). In contrast, biotinylated Neutrokine-alpha bound peripheral $CD20^+$ B cells. Receptor expression was also deteeted on the B cell tumor lines REH, ARH-77, Raji, Namalwa, RPMI 8226, and IM-9 but not any of the myeloid-derived lines tested including THP-1, HL-60, K-562, and U-937. Representative flow cytometric profiles for the myeloma cell line IM-9 and the histioeytic line U-937 are shown in Figures 10F and 10G. Similar results were also obtained using a biologically active FLAG-tagged Neutrokine-alpha protein instead of the chemieally modified biotin-Neutrokine-alpha. Taken together, these results confirm that Neutrokine-alpha displays a clear B cell tropism in both its reeeptor distribution and biologieal aetivity. It remains to be shown whether cellular activation may induce expression of Neutrokine-alpha receptors on peripheral blood cells, other normal cell types or established cell lines.

[0985] To examine the species speeificity of Neutrokine-alpha, mouse splenic B eells were cultured in the presence of human Neutrokine-alpha and SAC. Results demonstrate that rNeutrokine-alpha indueed *in vitro* proliferation of murine splenic B cells and bound to a cell surface reeeptor on these cells. Interestingly, immature surface Ig negative B cell

precursors isolated from mouse bone marrow did not proliferate in response to Neutrokine-alpha nor did they bind the ligand.

[0986] To assess the *in vivo* activity of rNeutrokine-alpha, BALB/c mice (3/group) were injected (i.p.) twice per day with buffer only, or 0.08 mg/kg, 0.8 mg/kg, 2 mg/kg or 8 mg/kg of rNeutrokine-alpha. Mice reeeived this treatment for 4 consecutive days at which time they were sacrifieed and various tissues and serum collected for analyses. In an alternative embodiment, BALB/c mice may be injected (i.p.) twice per day with any amount of rNeutrokine-alpha in a range of 0.01 to 10 mg/kg. In a preferred embodiment, BALB/c mice are injected (i.p.) twice per day with any amount of rNeutrokine-alpha in a range of 0.01 to 3 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, and 3.0 mg/kg). In an additional preferred embodiment, BALB/c mice are injected (i.p.) twice per day with any amount of rNeutrokine-alpha in a range of 0.02 to 2 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, and 2.0 mg/kg).

[0987] Microscopically, the effects of Neutrokine-alpha administration were clearly evident in sections of spleen stained with routine hematoxylin and eosin (H&E) and immunohistochemically with a mAb speeific for CD45R(B220) (Figure 11A). Normal splenic architecture was altered by a dramatic expansion of the white pulp marginal zone and a distinct inerease in cellularity of the red pulp (Figure 11A). Marginal zone expansion appeared to be the result of increased numbers of lymphocytes expressing the B cell marker CD45R(B220). In addition, the T cell dense periarteriolar lymphoid sheath (PALS) areas were also infiltrated by moderate numbers of CD45R(B220) positive eells. This suggests the white pulp changes were due to increased numbers of B cells. The

densely packed cell population that frequently filled red pulps spaces did not stain with CD45R(B220). Additional experiments will be required to characterize all the cell types involved and further define the mechanism by which Neutrokine-alpha alters splenic architecture.

[0988] Flow cytometric analyses of the spleens from mice treated with 2 mg/kg Neutrokine-alpha-treated indicated that Neutrokine-alpha increased the proportion of mature ($CD45R(B220)^{dull}$, ThB^{bright}) B cells approximately 10-fold over that observed in control mice (Figures 11B and 11C). Further analyses performed in which mice were treated with buffer, 0.08 mg/kg, 0.8 mg/kg, 2 mg/kg, or 8 mg/kg Neutrokine-alpha indicated that 0.08 mg/kg, 0.8 mg/kg, and 2 mg/kg each increased the proportion of mature ($CD45R(B220)^{dull}$, ThB^{bright}) B cells approximately 10-fold over that observed in control mice, whereas buffer and 8 mg/kg produced approximately equal proportions of mature B cells. *See*, Table IV.

Table IV. FACS Analysis of Mouse Spleen B cell Population.

Neutrokine-alpha (mg/kg)	*% Mature B Cells (R2)*	*% CD45R-positive (R1)*
Control (buffer)	1.26	52.17
0.08 mg/kg	16.15	56.53
0.8 mg/kg	18.54	57.56
2 mg/kg	16.54	57.55
8 mg/kg	1.24	61.42

[0989] A potential consequence of increased mature B cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgA, IgG and IgM levels were compared between buffer and Neutrokine-alpha-treated mice (Figures 11D, 11E, and 11F). Neutrokine-alpha administration resulted in a 2- and 5-fold increase in IgA and IgM serum levels respectively. Interestingly, circulating levels of IgG did not increase.

[0990] Moreover, a dose-dependent response was observed in serum IgA titers in mice treated with various amounts of Neutrokine-alpha over a period of four days, whereas no apparent dose-dependancy was observed by administration of the same amounts of Neutrokine-alpha over a period of two days. In the case of administration over four days, administration of 8, 2, 0.8, 0.08, and 0 mg/kg Neutrokine-alpha resulted in serum IgA

titers of approximately 800 micrograms/ml, 700 micrograms/ml, 400 micrograms/ml, 200 micrograms/ml and 200 micrograms/ml. That is, administration of 8, 2, 0.8, and 0.08 mg/kg Neutrokine-alpha over four days resulted in approximately 4-fold, 3.75-fold, 2-fold, and minimal-fold, respectively, increases in IgA serum levels over background or basal levels observed by administration of buffer only. In an alternative embodiment, these experiments may be performed with any amount of rNeutrokine-alpha in a range of 0.01 to 10 mg/kg. In a preferred embodiment, Neutrokine-alpha is administered in a range of 0.01 to 3 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, and 3.0 mg/kg). In an additional preferred embodiment, Neutrokine-alpha is administered in a range of 0.02 to 2 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, and 2.0 mg/kg).

[0991] The data presented herein define Neutrokine-alpha, as a novel member of the TNF-ligand superfamily that induces both *in vivo* and *in vitro* B cell proliferation and differentiation. Neutrokine-alpha is distinguished from other B cell growth and differentiation factors such as IL2 (Metzger, D.W., *et al., Res.Immunol.* 146:499-505 (1995)), IL4 (Armitage, R.J., *et al., Adv.Exp.Med.Biol.* 292:121-30 (1991); Yokota, T., *et al., Proc.Natl.Acad.Sci.U.S.A.* 83:5894-98 (1986)), IL5 (Takatsu, K., *et al., Proc.Natl.Acad.Sci.U.S.A.* 84:4234-38 (1987); Bertolini, J.N., *et al., Eur.J.Immunol.* 23:398-402 (1993)), IL6 (Poupart, P., *et al., EMBO J.* 6:1219-24 (1987); Hirano, T., *et al., Nature* 324:73-76 (1986)) IL7 (Goodwin, R.G., *et al., Proc. Natl. Acad. Sci. U.S.A.* 86:302-06 (1989); Namen, A.E., *et al., Nature* 333:571-73 (1988)), IL13 (Punnonen, J., *et al., Allergy.* 49:576-86 (1994)), IL15 (Armitage, R.J., *et al., J.Immunol.* 154:483-90 (1995)), CD40L (Armitage, R.J., *et al., Nature* 357:80-82 (1992); Van Kooten, C. and

Banchereau, J. *Int.Arch.Allergy.Immunol.* 113:393-99 (1997)) or CD27L (CD70) (Oshima, H., *et al., Int.Immunol.* 10:517-26 (1998); Lens, S.M., *et al., Semin.Immunol.* 10:491-99 (1998)) by its monocyte-specific gene/protein expression pattern and its specific receptor distribution and biological activity on B lymphocytes. Taken together these data suggest that Neutrokine-alpha is likely involved in the exchange of signals between B cells and monocytes or their differentiated progeny. Although all B cells may utilize this mode of signaling, the restricted expression patterns and Ig secretion suggest a role for Neutrokine-alpha in the activation of $CD5^+$ or "unconventional" B cell responses. These B cells provide a critical component to the innate immune system and provide protection from environmental pathogens through their secretion of polyreactive IgM and IgA antibodies (Pennell, C.A., *et al., Eur.J.Immunol.* 19:1289-95 (1989); Hayakawa, K., *et al., Proc.Natl.Acad.Sci.U.S.A.* 81:2494-98 (1984)). Alternatively, Neutrokine-alpha may function as a regulator of T cell independent responses in a manner analogous to that of CD40 and CD40L in T cell dependent antigen activation (van den Eertwegh, A.J., *et al., J.Exp.Med.* 178:1555-65 (1993); Grabstein, K.H., *et al., J.Immunol.* 150:3141-47 (1993)). As sueh, Neutrokine-alpha, its reeeptor or related antagonists have utility in the treatment of B cell disorders associated with autoimmunity, neoplasia and/or immunodeficient syndromes.

Methods

[0992] Mice. BALB/cAnNCR (6-8 weeks) were purchased from Charles River Laboratories, Inc. and maintained according to recommended standards (National Research Couneil, *Guide for the care and use of laboratory animals* (1999)) in microisolator cages with recycled paper bedding (Harlan Sprague Dawley, Ine., Indianapolis, IN) and provided with pelleted rodent diet (Harlan Sprague Dawley, Inc) and bottled drinking water on an ad libitum basis. The animal protocols used in this study were reviewed and approved by the HGS Institutional Animal Care and Use Committee.

[0993] Isolation of full length Neutrokine-alpha cDNA. The BLAST algorithm was used to search the Human Genome Sciences Inc. expressed sequence tag (EST) database for sequences with homology to the receptor-binding domain of the TNF family. A full length Neutrokine-alpha clone was identified, sequenced and submitted to GenBank (Accession number AF132600). The Neutrokine-alpha open reading frame was PCR

amplified utilizing a 5' primer (5'-CAG ACT GGA TCC GCC ACC ATG GAT GAC TCC ACA GAA AG-3') annealing at the predicted start codon and a 3' primer (5'-CAG ACT GGT ACC GTC CTG CGT GCA CTA CAT GGC-3') designed to anneal at the predieted downstream stop codon. The resulting amplicon was tailed with Bam HI and Asp 718 restriction sites and subcloned into a mammalian expression vector. Neutrokine-alpha was also expressed in p-CMV-1 (Sigma Chemicals).

[0994] Purification of recombinant human Neutrokine-alpha. The full length cDNA encoding Neutrokine-alpha was subcloned into the baculovirus expression vector pA2 and transfected into Sf9 insect cells (Patel, V.P., *et al., J.Exp.Med.* 185:1163-72 (1997)). Recombinant Neutrokine-alpha was purified from cell supernatants at 92 h post-infection using a combination of anion-exchange, size exclusion, and hydrophobic interaction eolumns. The purified protein was formulated in a buffer containing 0.15 M NaCl, 50 mM NaOAc at pH 6, sterile filtered and stored at 4°C until needed. Both SDS-PAGE and RP-HPLC analyses indicate that rNeutrokine-alpha is greater than 95% pure. Endotoxin levels were below the detection limit in the LAL assay (Assoeiates of Cape Cod, Falmouth, MA). The final purified Neutrokine-alpha protein has an N-terminus sequence of Ala-Val-Gln-Gly-Pro. This corresponds identically to the sequence of soluble Neutrokine-alpha derived from CHO cell lines stably transfeeted with the full length Neutrokine-alpha gene.

[0995] Monoclonal antibody generation. BALB/cAnNCR mice were immunized with 50 micrograms of HisTag-Neutrokine-alpha suspended in complete Freund's adjuvant followed by 2 challenges in incomplete Freund's adjuvant. Hybridomas and monoclonal antibodies were prepared as described (Gefter, M.L., *et al., Somatic.Cell Genet.* 3:231-36 (1977); Akerstrom, B., *et al., J.Immunol.* 135:2589-92 (1985)).

[0996] Cell lines. All human cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA).

[0997] FACS analysis. Neutrokine-alpha expression was assessed on human cell lines, freshly isolated normal peripheral blood nucleated cells, and in vitro cultured monocytes, a mouse anti-human Neutrokine-alpha mAb 2E5 (IgG1) followed by PE-conjugated F(ab')2 goat antibody to mouse IgG (CALTAG Laboratories, Burlingame, CA). Cells were analyzed using a FACSean (Becton Dickinson Immunocytometry Systems, San Jose, CA) with propidium iodide to exclude dead cells. Neutrokine-alpha

binding was assessed using rNeutrokine-alpha biotinylated with a N-hydroxysuccinimidobiotin reagent (Pierce, Rockford, IL) followed by PE-conjugated streptavidin (Dako Corp, Glostrup, Denmark).

[0998] Chromosomal mapping. To determine the chromosomal location of the Neutrokine-alpha gene, a panel of monochromosomal somatic cell hybrids (Quantum Biotechnology, Canada) retaining individual chromosomes was screened by PCR using Neutrokine-alpha specific primers (5' primer: 5'-TGG TGT CTT TCT ACC AGG TGG-3' and 3' primer: 5'-TTT CTT CTG GAC CCT GAA CGG-3'). The predicted 233 bp PCR product was only detected in human chromosome 13 hybrids. Using a panel of 83 radiation hybrids (Research Geneties, St. Louis, MO) and the Stanford Human Genome Center Database, (http://www.shgc.stanford.edu.RH/rhserver). Neutrokine-alpha was found linked to the SHGC-36171 marker on chromosome 13. Superposition of this map with the cytogenetic map of human chromosome 13 allowed the assignment of human Neutrokine-alpha to chromosomal band 13q34.

[0999] B lymphocyte proliferation assay. Human tonsillar B cells were purified by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population was routinely greater than 95% B cells as assessed by expression of CD19 and CD20. Various dilutions of human rNeutrokine-alpha or the control protein recombinant human IL2 were placed into individual wells of a 96-well plate to whieh was added 10^5 B cells suspended in culture medium (RPMI 1640 eontaining 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 100 microgram/ml streptomycin, and 10^{-5} dilution of Pansorbin (SAC) or anti-IgM) in a total volume of 150 microliters. Proliferation was quantitated by a 20h pulse (1 microCi/well) of ^{3}H-thymidine (6.7 Ci/mM) beginning 72h post factor addition.

[1000] Histological analyses. Spleens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 micrometers, mounted on glass slides and stained with hematoxylin and eosin or by enzyme-labeled indirect method immunohistochemistry for CD45R(B220) (Hilbert, D.M., *et al., Eur.J.Immunol.* 23:2412-18 (1993)).

Table V. Neutrokine-alpha cell surface expression

Cell line	Cellular Morphology	Neutrokine-alpha cell surface expression

Monocytic lineage

U-937	Lymphoma, histiocytic/macrophage	+
BL-60	Leukemia, acutepromyelocytic	+
K-562	Leukemia, chronlcmyelogenous	+
THP-1	Leukemia, acutemonocytic	+

T-lineage

Jurkat	Leukemia, T lymphocytic	-
SUP-T13	Leukemia, T lymphoblastic	-
MOLT-4	Leukemia, T lymphoblastic	-

B-lineage

Daudi	Burkitt's, lymphoblastie	-
Namalwa	Burkitt's, lymphocyte	-
Raji	Burkitt's, lymphocyte	-
Reh	Leukemia, lymphocytic	-
ARH-77	Leukemia, plasma cell	-
IM9	Myeloma	-
RPMI 8226	Myeloma	-

Example 7: Assays to detect stimulation or inhibition of B cell proliferation and differentiation

[1001] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their mierocnvironment. Signals may impart a positive stimulus that allows a B-lineage eell to continue its programmed development, or a negative stimulus that instruets the eell to arrest its eurrent developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL5, IL6, IL-7, IL10, IL-13, IL14 and IL15. Intcrestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations. One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell

populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

[1002] *In Vitro* assay- Purified Neutrokine-alpha and/or Neutrokine-alphaSV protein, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of Neutrokine-alpha and/or Neutrokine-alphaSV protein on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220). Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10 ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with ^{3}H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

[1003] Agonists (including Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide fragments) demonstrate an increased B cell proliferation when compared to that observed when the same number of B cells is contacted with the same concentration of priming agent. Antagonists according to the invention exhibit a decreased B cell proliferation when compared to controls containing the same number of B cells, the same concentration of priming agent, and the same concentration of a soluble form of Neutrokine-alpha that elicits an increase in B cell proliferative activity (e.g., 71-285, 81-285, 112-285 or 134-285 of the Neutrokine-alpha polypeptide shown in SEQ ID NO:2) in the absence the antagonist.

[1004] *In Vivo* assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of Neutrokine-alpha and/or Neutrokine-alphaSV protein, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal and Neutrokine-alpha and/or Neutrokine-alphaSV protein-treated spleens identify the results of the aetivity of Neutrokine-alpha and/or Neutrokine-alphaSV protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or signifieant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-eell regions.

[1005] Flow cytometric analyses of the spleens from Neutrokine-alpha and/or Neutrokine-alphaSV protein-treated mice is used to indicate whether Neutrokine-alpha and/or Neutrokine-alphaSV protein specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

[1006] Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative inerease in serum Ig titers. Accordingly, serum IgM and IgA levels are eompared between buffer and Neutrokine-alpha and/or Neutrokine-alphaSV protein-treated mice.

Example 8: Effect of Neutrokine-alpha and its agonists in treating graft-versus-host disease associated lymphoid atrophy and hypoplasia in mice

[1007] An analysis of the use of Neutrokine-alpha to treat, prevent, and/or diagnose graft-versus-host disease (GVHD)-associated lymphoid hypoplasia/atrophy is performed through the use of a C57BL/6 parent into (BALB/c X C57BL/6) F1 (CBF1) mouse model. This parent into F1 mouse model is a well-characterized and reproducible animal model of GVHD in bone marrow transplant patients, which is well know to one of ordinary skill in the art (see, Gleichemann, *et al., Immunol. Today* 5:324, 1984). Soluble Neutrokine-alpha is expected to induced the proliferation and differentiation of B lymphoeyte, and correct

the lymphoid hypoplasia and atrophy observed in this animal model of GVHD (Piguet, *et al., J. Exp. Med.* 166:1280 (1987); Hattori, *et al.*, Blood 90:542 (1997)).

[1008] Initiation of the GVHD condition is induced by the intravenous injection of approximately 1-5 x 10^8 spleen cells from C57BL/6 mice into (BALB/c X C57BL/6) F1 mice (both are available from Jackson Lab, Bar Harbor, Maine). Groups of 6 to 8 mice receive daily either 0.1 to 5.0 mg/kg of Neutrokine-alpha or buffer control intraperitoneally, intramascullarly or intradermally starting from the days when lymphoid hypoplasia and atrophy are mild (~day 5), moderate (~day 12) or severe (~day 20) following the parental cell injection. The effect of Neutrokine-alpha on lymphoid hypoplasia and atrophy of spleen is analyzed by FACS and histopathology at multiple time points (3-4) between day 10-30. Briefly, splenocytes are prepared from normal CBF1, GVHD or Neutrokine-alpha-treated mice, and stained with fluorescein phycoerythrin-conjugated anti- H-2Kb, biotin-conjugated anti- H-2Kd, and FITC-conjugated anti-CD4, anti-CD8, or anti-B220, followed by a CyChrome-conjugated avidin. All of these conjugated antibodies can be purchased from PharMingen (San Diego, CA). Cells are then analysis on a FACScan (Becton Dickinson, San Jose, CA). Recipient and donor lymphocytes are identified as H-2Kb+ Kd+ and H-2Kb+ Kd- cells, respectively. Cell numbers of CD4+T, CD8+ T and B220+ B cells of recipient or donor origin are calculated from the total numbers of splenocytes recovered and the percentages of each subpopulation are determined by the three color analysis. Histological evaluation of the relative degree of tissue damage in other GVHD-associated organs (liver, skin and intestine) may be conducted after sacrificing the animals.

[1009] Finally, Neutrokine-alpha and buffer-treated animals undergo a clinical evaluation every other day to assess cachexia, body weight and lethality.

[1010] Neutrokine-alpha agonists and antagonists may also be examed in this acute GVHD murine model.

Example 9. Isolation of antibody fragments directed against Neutrokine-alpha polypeptides from a library of scFvs.

[1011] Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against Neutrokine-alpha

and/or Neutrokine-alphaSV to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

Rescue of the library.

[1012] A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047 (which is hereby incorporated by reference in its entirety). To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used to inoculate 50 ml of 2x TY containing 1% glucose and 100 micrograms/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10^8 TU of delta gene 3 helper (M13 delta gene III, see WO92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2x TY containing 100 micrograms/ml ampicillin and 50 micrograms/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[1013] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells were spun down (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2x TY broth containing 100 micrograms ampicillin/ml and 25 micrograms kanamycin/ml (2x TY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 micrometer filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

Panning the Library.

[1014] Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 micrograms/ml or 10 micrograms/ml of a polypeptide of the present invention. Tubes are

blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10^{13} TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100 mierograms/ml ampieillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders.

[1015] Eluted phage from the third and fourth rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single eolonies for assay. ELISAs are performed with microtiter plates coated with either 10 picograms/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

Example 10. Neutralization of Neutrokine-alpha/Neutrokine-alpha Receptor Interaction with an anti-Neutrokine-alpha Monoclonal Antibody.

[1016] Monoelonal antibodies were generated against Neutrokine-alpha protein aceording to the following method. Briefly, miee were given a subcutaneous injection (front part of the dorsum) of 50 micrograms of His-tagged Neutrokine-alpha protein produced by the method of Example 2 in 100 mieroliters of PBS emulsified in 100 mieroliters of complete Freunds adjuvant. Three additional subcutaneous injections of 25 micrograms of Neutrokine-alpha in incomplete Freunds adjuvant were given at 2-week intervals. The animals were rested for a mounth before they received the final

intraperitoneal boost of 25 micrograms of Neutrokine-alpha in PBS. Four days later mice were sacrificed and splenoeytes taken for fusion.

[1017] The process of "Fusion" was accomplished by fusing splenocytes from one spleen were with 2x10E7 P3X63Ag8.653 plasmacytoma cells using PEG 1500 (Boehringer Mannheim), aceording to the manufacturer's modifications of an earlier described method. (*See*, Gefter, M.L., *et al. Somatic Cell Genet* 3:231-36 (1977); Boehringer Mannheim, PEG 1500 (Cat.No. 783641), product description.)

[1018] After fusion, the cells were resuspended in 400 ml of HAT medium supplemented with 20% FBS and 4% Hybridoma Supplement (Boehringer Mannheim) and distributed to 96 well plates at a density of 200 microliters per well. At day 7 post-fusion, 100 mieroliters of medium was aspirated and replaced with 100 microliters of fresh medium. At day 14 post-fusion, the hybridomas were screened for antibody produetion.

[1019] Hybridoma supernatants were screened by ELISA for binding to Neutrokine-alpha protein immobilized on plates. Plates were coated with Neutrokine-alpha by overnight incubation of 100 microliters per well of Neutrokine-alpha in PBS at a concentration of 2 micrograms per ml. Hybridoma supernatants were diluted 1:10 with PBS were placed in individual wells of Neutrokine-alpha-coated plates and ineubated overnight at 4°C. On the following day, the plates were washed 3 times with PBS containing 0.1% Tween-20 and developed using the anti-mouse IgG ABC system (Vector Laboratories). The color development reaction was stopped with the addition of 25 ml/well of 2M H_2SO_4. The plates were then read at 450 nm.

[1020] Hybridoma supernatants were checked for Ig isotype using Isostrips. Cloning was done by the method of limiting dilutions on HT medium. About 3x10E6 cells in 0.9 ml of HBSS were injected in pristane-primed mice. After 7-9 days, ascitic fluid was collected using a 19 g needle. All antibodies were purified by protein G affinity chromatography using the Acta FPLC system (Pharmaeia).

[1021] After primary and two consecutive subcutaneous injections, all three miee developed a strong immune response; the serum titer was 10E-7 as assessed by ELISA on Neutrokine-alpha-coated plates.

[1022] In one experiment, using the splenocytes from the positive mouse more than 1000 primary hybridomas were generated. 917 of them were screened for producing anti-Neutrokine-alpha antibody. Sereening was performed using 1:1 diluted supernatants in

order to detect all positive clones. Of 917 hybridomas screened, 76 were found to be positive and 17 of those were found to be IgG producers. After affinity testing and cloning, 9 of them were chosen for further expansion and purification.

[1023] All purified monoclonal antibodies were able to bind different forms of Neutrokine-alpha (including His-tagged and protein produced from a baculoviral system (see Example 2)) in both Western blot analysis and ELISA. Six of nine clones were also able to bind Neutrokine-alpha on the surface of THP-1 cells. However, none of the antibodies tested were able to capture Neutrokine-alpha from solution.

[1024] High affinity anti-Neutrokine-alpha monoclonal antibodies were generated that recognize Neutrokine-alpha expressed on the cell surface but not in solution can be used for neutralization studies *in vivo* and in monocyte and B cell assays *in vitro*. These antibodies are also useful for sensitive detection of Neutrokine-alpha on Western blots.

[1025] In an independent experiment, using the splenocytes from the positive mouse, more than 1000 primary hybridomas were generated. 729 of the primary hybridomas were then screened for the production of an anti-Neutrokine-alpha antibody. Screening was performed under stringent conditions using 1:10 diluted supernatants in order to pick up only clones of higher affinity. Of 729 hybridomas screened, 23 were positive, including 16 IgM and 7 IgG producers (among the latter, 4 gave a strong IgM background). In this experiment, the isotype distribution of IgG antibodies was biased towards the IgG2 subclasses. Three of seven IgG hybridomas produced antibodies of IgG2a subclass and two produced an antibody of IgG2b subclass, while the remaining two were IgG1 producers.

[1026] Supernatants from all positive hybridomas generated in the second experiment were tested for the ability to inhibit Neutrokine-alpha-mediated proliferation of B cells. In the first screening experiment, two hybridomas producing IgG-neutralizing antibodies were detected (these are antibodies 16C9 and 12C5). In additional experiments, the IgG-neutralizing activity of the hybridomas (i.e., 16C9 and 12C5) were confirmed and two additional strongly neutralizing supernatants from hybridomas 15C10 and 4A6 were indentified.

[1027] Three clones were subsequently expanded *in vivo* (a single clone, i.e., 15C10, was also expanded in a hollow fiber system), and the antibody purified by affinity chromatography. All three of the clones were able to bind Neutrokine-alpha on the

surface of THP-1 eells and were also able to bind (i.e., "capture") Neutrokine-alpha from solution.

[1028] Specifieally, experiments were performed using the anti-Neutrokine-alpha monoclonal antibodies deseribed in the second experiment above to determine whether the antibodies neutralize Neutrokine-alpha/Neutrokine-alpha Receptor binding. Briefly, Neutrokine-alpha protein was biotinylated using the EZ-link T NHS-biotin reagent (Pierce, Rockford, IL). Biotinylated Neutrokine-alpha was then used to identify cell surface proteins that bind Neutrokine-alpha. Preliminary experiments demonstrated that Neutrokine-alpha binds to a receptor on B lymphoid cells.

[1029] The inelusion of anti-Neutrokine-alpha antibodies generated in the second experiment described above neutralized binding of Neutrokine-alpha to a Neutrokine-alpha receptor. In a specific embodiment, anti-Neutrokine-alpha antibody 15C10 neutralizes binding of Neutrokine-alpha to a Neutrokine-alpha Receptor.

[1030] Thus, the anti-Neutrokine-alpha monoclonal antibodies generated in the second experiment described above (in particular, antibody 15C10) recognize and bind to both membrane-bound and soluble Neutrokine-alpha protein and neutralize Neutrokine-alpha/Neutrokine-alpha Receptor binding *in vitro*.

Example 11. Neutrokine-alpha induced signalling in B cells

[1031] Total RNA was prepared from tonsillar B cells unstimulated or stimulated with SAC or SAC plus soluble Neutrokine-alpha (amino acids 134-285 of SEQ ID NO:2, 100ng/mL) for 12 hours. Messenger RNA levels of ERK-1 and PLK was determined by real time quantitaive PCR using ABI 7700 Taqman sequence detector. Amplification primers and probes were designed to span the region from nucleotides 252-332 of the human PLK sequenee and nucleotides 373 to 446 of the human ERK-1 mRNA (Genbank accession numbers X75932 and X60188, respeetively). For quantitation of RNA, the comparative delta CT method was used (Perkin-Elmer user Bulletin #2 and #4, 1997) using an 18S ribosomal RNA probeas endogenous reference. Expression levels were characterized relative to observed levels in unstimulated B-cells.

Example 12. Rapid and Specific Targeting of Radiolabeled Neutrokine-alpha to Lymphoid Tissues

[1032] Here, biodistribution studies of radiolabeled Neutrokine-alpha are reported that demonstrate high *in vivo* targeting specificity of Neutrokine-alpha for lymphoid tissues. Neutrokine-alpha was radiolabeled with ^{125}I and injected intravenously into BALB/c mice. Three doses and 4 timepoints over a 24-hr period were studied. Biodistribution was measured by direct counting of the radioaetivity in dissected whole organs or tissues and by whole body quantitative autoradiography (QAR).

[1033] Spleen and lymph nodes showed the highest concentration of radioactivity among the dissected organs and tissues. Three hr after injection of 0.01 mg/kg Neutrokine-alpha, 63% and 23% injeeted dose (ID)/g were measured in spleen and lymph node, respeetively, compared to ~5% for both kidney and liver. As the dose was increased, the %ID/g in spleen and lymph node decreased but was unchanged in liver and kidney, suggesting that targeting to spleen and lymph nodes is mediated by saturable binding. With increasing time, the ratio of the concentration in spleen and lymph node to the concentration in either kidney or liver increased. QAR confirmed the high uptake of radiolabeled Neutrokine-alpha in spleen and lymph nodes at 3 hr, and revealed high uptake in bone marrow, gut-associated lymphoid tissue (GALT) and intestinal contents as well. At 24 hr, spleen, lymph nodes and GALT were still strongly positive for radiolabeled Neutrokine-alpha by QAR whereas liver and kidney no longer had observable levels. A cytotoxic radionuclide coupled to Neutrokine-alpha could irradiate neoplastic B-cells trafficking through or residing in lymphoid tissues. Thus, the rapid and highly specific targeting of radiolabeled Neutrokine-alpha to lymphoid tissues provides a rationale for its application in the treatment of B-eell malignaneies.

Example 13: Pharmacological Effects of ^{131}I-labeled Neutrokine-alpha in BCL1 Tumor-Bearing Mice and J558 Tumor Bearing Mice

^{131}I-Neutrokine-alpha administration to BCL1 Tumor-Bearing Mice

[1034] The BCL1 tumor cell line was derived from a spontaneous murine B cell tumor. Intraperitoneal inoculation of the BCL1 cell line in BALB/c mice results in splenomegaly, and subsequent death. The BCL1 tumor cell phenotype is IgM positive, complement receptor negative, Fe reeeptor positive and has marginal IgD expression (Knapp et al., J. Immunol. 123:992-999 (1979) and Vitetta et al Blood 89:4425-36.

(1997)). Based on FACS analysis using biotinylated Neutrokine-alpha, BCL1 cells freshly isolated from the spleens of BALB/c mice express Neutrokine-alpha receptors on their cell surface. The BCL1 tumor model is a relevant mouse model for human B cell lymphoma, providing a means to test the ability of ^{131}I-labeled Neutrokine-alpha to kill leukemic B cells and consequently prolong survival of tumor-bearing mice. Three lots of ^{131}I-labeled Neutrokine-alpha (Lots TX1, TX2 and TX3) were prepared by MDS Nordion (Ontario, Canada) and used in 3 different experiments to evaluate the effects of ^{131}I-labeled Neutrokine-alpha in this murine model.

[1035] Female BALB/c mice were injected intraperitoneally (ip) on Day 0 with 1 x 10^5 viable BCL1 cells that had been propagated in vivo. Treatment groups for the 3 experiments are described in Table VI. Ten days after injection of tumor cells, the animals were administered ^{131}I-labeled Neutrokine-alpha iv in 110 µL. The doses administered were 11.9 or 15.3 mCi/kg (TX1), 17.5 mCi/kg (TX2), or 37.7 mCi/kg (TX3) for the 3 experiments. To identify potentially toxic effects of the administered ^{131}I-labeled Neutrokine-alpha, age-matched control BALB/c mice without BCL1 tumors were injected with identical doses of the ^{131}I-labeled protein. An additional group of BALB/c mice, bearing BCL1 tumors and receiving an iv injection of the vehicle, served as the normal tumor control group. Survival was then monitored for 48, 44, or 40 days for the TX1, TX2, and TX3 experiments, respectively.

Table VI Treatment groups for TX1, TX2 and TX3 experiments

Exp.	Group	^{131}I-Neutrokine-alpha Dose (mCi/kg)	n	BCL1 Tumor Inoculated ip (No. of cells)
1 (TX1)	1 Vehicle	0	15	1 x 10^5
	2 ^{131}I-Neutrokine-alpha	11.9	10	1 x 10^5
	3 ^{131}I-Neutrokine-alpha	15.3	10	1 x 10^5
	4 ^{131}I-Neutrokine-alpha	11.9	10	0
	5 ^{131}I-Neutrokine-alpha	15.3	10	0
2 (TX2)	1 Vehicle	0	12	1 x 10^5
	2 ^{131}I-Neutrokine-alpha	17.5	12	1 x 10^5
	3 ^{131}I-Neutrokine-alpha	17.5	8	0
3 (TX3)	1 Vehicle	0	14	1 x 10^5
	2 ^{131}I-Neutrokine-alpha	37.7	14	1 x 10^5
	3 ^{131}I-Neutrokine-alpha	37.7	8	0

[1036] The endpoint monitored in the 3 experiments was survival (days) following ip inoculation of BCL1 tumor cells. All animals were examined daily. The day post-inoculation that miee were either found dead or in moribund condition (the latter being immediately euthanized for humane reasons) was recorded.

[1037] A single iv administration of either 11.9 or 15.3 mCi/kg (TX1), 17.5 mCi/kg (TX2), or 37.7 mCi/kg (TX3) of ^{131}I-labeled Neutrokine-alpha injected 10 days after intraperitoneal inoculation of BCL1 cells in BALB/c mice significantly improved survival compared with mice inoculated with tumor and treated with the ^{131}I-labeled Neutrokine-alpha vehicle (Figures 12-14; in Figures 12-14, ^{131}I-labeled Neutrokine-alpha is indicated as LR131). The median survival time for the vehicle-treated, tumor-bearing mice was 18, 21, and 19 days post-tumor cell injection for the TX1, TX2, and TX3 experiments, respectively. In the TX1 experiment, ^{131}I-labeled Neutrokine-alpha administration at dose levels of 11.9 and 15.3 mCi/kg doubled the median survival time of tumor-bearing mice to 35.5 (11.9 mCi/kg) and 34 (15.3 mCi/kg) days post-treatment, respectively. In the TX2 and TX3 experiments, ^{131}I-labeled Neutrokine-alpha administration at a dose of 17.5 or 37.7 mCi/kg increased the median survival time of tumor-bearing miee to 30 and 22 days post-treatment, respectively. Tumor-bearing mice treated with all doses of ^{131}I-labeled Neutrokine-alpha in the 3 experiments had a significantly lower risk of dying than tumor-bearing miee treated with vehicle (Table VII).

Table VII Incidence of mortality for TX1 – TX3 experiments

Experiment	Treatment Group	Median Survival Time (Days)
TX1	1, BCL1 + ^{131}I-labeled Neutrokine-alpha (11.9 mCi/kg)	35.5
	2, BCL1 + ^{131}I-labeled Neutrokine-alpha (15.3 mCi/kg)	34
	3, BCL1 Tumor Only	18
	4, No Tumor + ^{131}I-labeled Neutrokine-alpha (11.9 mCi/kg)	> 48
	5, No Tumor + ^{131}I-labeled Neutrokine-alpha (15.3 mCi/kg)	> 48
TX2	1, BCL1 + ^{131}I-labeled Neutrokine-alpha (17.5 mCi/kg)	30
	2, BCL1 Tumor Only + vehicle	21
	3, No Tumor + ^{131}I-labeled Neutrokine-alpha (17.5 mCi/kg)	> 44
TX3	1, BCL1 + vehicle	19
	2, BCL1 + ^{131}I-labeled Neutrokine-alpha (37.7 mCi/kg)	22
	3, No tumor + ^{131}I-labeled Neutrokine-alpha (37.7 mCi/kg)	> 40

[1038] In the TX1 – TX3 series of experiments, the effect that increasing the dose of ^{131}I-labeled Neutrokine-alpha had on the survival of the BCL1 tumor-bearing animals was investigated. A maximal survival benefit was achieved with the low doses of ^{131}I-labeled Neutrokine-alpha (11.9 and 15.3 mCi/kg). The much redueed effectiveness of ^{131}I-labeled Neutrokine-alpha in TX3 may be due to toxicity associated with the high dose of the material used.

[1039] In conclusion, a single iv administration of ^{131}I-labeled Neutrokine-alpha administered to mice bearing BCL1 leukemia cell splenic tumors significantly improved survival compared with tumor-bearing mice treated with vehicle.

^{131}I-Neutrokine-alpha administration to J558 Tumor-Bearing Mice

[1040] In a similar experiment as that described above, BALB/c mice were injected subcutaneously with J558 plasmaeytoma cells (ATCC # TIB-6) and treated with a single intravenous treatment of 25mCi/kg of ^{131}I-labeled Neutrokine-alpha. 24 BALB/c mice (NCI, 4 weeks old, average weight 18 g) were divided into 2 groups (12 mice per group) and injected sc with 2.5 x 10^5 J558 cells in 100 mL of PBS. At Day 9 after injection, mice in Group 1 were injected intavenously with 100 mL of formulation buffer, and mice in Group 2 were injected iv with a dose of 25 mCi/kg of ^{131}I-Neutrokine-alpha in 100 mL of formulation buffer. The average body weight at the time of ^{131}I-Neutrokine-alpha injection was 19.5 g.

[1041] Two parameters were evaluated during this study the tumor size and the time to tumor response. To evaluate tumor size the short and long axes of the tumor were measured using an electronic digital caliper. Tumor size was calculated by multiplication of the lengths of the short and long axes and expressed in mm^2. The time to tumor response was characterized by the day after cell inoculation when a visible tumor (> 2mm) was detected on a mouse. In addition, mice were monitored for survival and signs of radiation induced toxicity (general appearance, activity, breathing frequency, stool consistence).

[1042] One mouse in the ^{131}I-Neutrokine-alpha-treated group died on Day 25 (16 days after ^{131}I-Neutrokine-alpha treatment) with no obvious signs of radiation related toxicity.

A second mouse died in the same group on Day 30, when all animals in the control group were terminated because of large tumor size.

[1043] The first tumors of measurable size were detected at Day 14 in the buffer control group, where 4 out of 12 animals developed tumors. In the ^{131}I-Neutrokine-alpha treated animals, tumor formation was delayed by 6 days. Only one mouse out of 12 developed a tumor at Day 20. At Day 22, there was only one tumor-bearing mouse in the ^{131}I-Neutrokine-alpha treated group out of 12 animals, whereas in the buffer control group, 11 out of 12 mice developed tumors of different sizes. At Day 27, the mean tumor size in the buffer control group was 489 mm^2 (all tumor positive mice in this group were terminated at this time point). In the ^{131}I-Neutrokine-alpha treated group, the mean tumor size was 32.7 mm2, 15 times smaller than in the buffer control group. Taken together, these data suggest a strong inhibition of J558 tumor development in mice treated with ^{131}I-Neutrokine-alpha at a dose of 25 mCi/kg and tumor load of 2.5 x 10^5 cells/mouse.

[1044] In conclusion, a single intravenous administration of ^{131}I-Neutrokine-alpha into BALB/c mice at a dose of 25 mCi/kg significantly inhibits subcutaneous growth of J558 plasma cell tumors. At the initial tumor load of 2.5 x 105 cells/mouse, a 6 day delay in tumor formation and a 15-fold reduetion in tumor size was observed in ^{131}I-Neutrokine-alpha treated animals.

[1045] The anti-neoplastie effects of ^{131}I-Neutrokine-alpha were accompanied by the expected B lymphocyte hypoplasia and a transient (<20 days) depletion of cKit$^+$ bone marrow precursors and peripheral platelets. Peripheral neutrophil, red blood cell, and monocyte counts were unaffected by ^{131}I-Neutrokine-alpha treatment. Taken together, the results demonstrate that ^{131}I-Neutrokine-alpha inhibits in vivo tumor growth in two models of B cell neoplasia. Moreover, ^{131}I-Neutrokine-alpha efficacy was not accompanied by significant bone marrow toxicities or peripheral myelosuppression.

EXAMPLE 14: Improved Method for Producing Neutrokine-alpha Using a Stringent Promoter and Low Expression Level

[1046] Neutrokine-alpha has been produced in *Escherichia coli* K-12 from the periplasmic fraction of the cell lysate. Using this system, soluble, properly folded, active Neutrokine-alpha is not obtainable from simple shake flask experiments. Yields of

soluble Neutrokine-alpha from complex media fermentations in small and large-scale bioreactors are on the order of 1-5 mg/L. Greater yields (25-38 mg/L) of soluble, properly folded, active Neutrokine-alpha can be accomplished in bioreaetors at low to medium cell density under defined medium conditions. Moreover, this low quantity of protein is difficult to purify via conventional methods.

[1047] This example describes a method for the production of high yields of soluble, properly folded, active Neutrokine-alpha in the periplasm of *Escherichia coli*, which permits the use of conventional methods for Neutrokine-alpha purifieation, such as those described below or in Example 2 (paragraphs [0938] -[0948] , with modifieations for *E. coli*, as would be apparent to one of ordinary skill in the art). Additionally, Neutrokine-alpha protein may be purified using affinity columns comprising Neutrokine-alpha binding peptides such as those described in WO 02/02641, which is herein incorporated by referenee in its entirety. Purified Neutrokine-alpha may be quantified using RP-HPLC.

[1048] This method relies on the expression of Neutrokine-alpha protein from the bacterial *phoA* promoter. The *phoA* promoter is a very tightly regulated system that exhibits a very low level of transcription in the presence of excess phosphate. As the phosphate level in the medium decreases below a threshold of ~4 mieromolar (Wanner, B.L., *J Cell Biochem* 51:47 (1993)), transcription is induced about 1000-fold. The *phoA* promoter yields a gradual build-up of reeombinant protein, instead of a sharp inerease of induction that occurs with other systems. This gradual or steady increase in reeombinant protein minimizes the chance of overwhelming the eomponents of the bacterial expression system and may also minimize the formation of inclusion bodies. Furthermore, this gradual build up permits the expression of proteins that might have been toxic to the eell if they were induced to high levels over a short period of time.

Expression Vector pML124

[1049] The expression vector, pML124, was created using pBR322 as the starting backbone. First, the endogenous NdeI site of pBR322 was eliminated by digesting it with NdeI, filling in the overhanging ends with the Klenow enzyme, then re-ligating the two blunt-ends back together (this created pML123). Next, pML123 was digested with EcoRI and BamHI restriction enzymes and the linear plasmid (loss of ~375 bp of DNA) was agarose gel purified (Qiagen).

[1050] The *phoA* promoter region was PCR-amplified from the *E. coli* K-12 chromosome (W3110; ATCC Catalogue No. 27325) with EcoRI (5') and BamHI (3') engineered sites. NdeI and KpnI sites were also engineered downstream of the *phoA* promoter to facilitate cloning of recombinant genes. Finally, the Shine-Dalgarno (SD) box was optimized for protein expression. The wild-type SD box and its adjacent sequence is as follows (the putative SD boxes are underlined and in bold):

5'-TTTGTACATGGAGAAAATAAA (SEQ ID NO:56):-[ATG, start of coding sequence]-3'

Optimized SD box and adjacent sequence is as follows:
5'-CACGTAAAGGAAGTATCTCAT (SEQ ID NO:57)-[ATG, start of coding sequence]-3'

[1051] The digested (EcoRI and BamHI) and purified *phoA* promoter PCR product was ligated into the agarose gel purified pML123 (described above). The ligation mixture was transformed into highly competent *E. coli* cells using standard techniques. Positive clones were identified via restriction analysis and DNA sequencing.

[1052] pML124 contains a gene for ampicillin resistance, a ColE1 replicon (pBR322-based), Rop, *phoA* promoter, the optimized Shine-Dalgarno (SD) box (above) and a multiple cloning site. Figure 15 is a plasmid map of pML124 and SEQ ID NO:52 is the nucleotide sequence of pML124. Additionally, plasmid pML124 was deposited at the American Type Culture Collection (ATCC) on October 8, 2001 and given ATCC Deposit No. PTA-3778. ATCC Deposit Nos. PTA-3778 was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The ATCC (American Type Culture Collection) is located at 10801 University Boulevard, Manassas, Virginia 20110-2209.

Neutrokine-alpha Expression Vector pML124-MBPssBLyS

[1053] A fusion construct of the maltose binding protein signal sequence (MBPss) and Neutrokine-alpha was placed behind the *phoA* promoter in pML124 as follows. A 549 bp NdeI/KpnI MBPss-Neutrokine-alpha containing DNA insert was ligated into NdeI/KpnI

digested and gel purified pML124 to form pML124-MBPss-BLyS. (Figures 16, SEQ ID NO:53 ATCC Deposit No. PTA-3867, deposited November 16, 2001). ATCC Deposit No. PTA-3867 was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The ATCC (American Type Culture Collection) is located at 10801 University Boulevard, Manassas, Virginia 20110-2209.

[1054] The pML124 plasmid (Figures 15, SEQ ID NO:52) is described in above and in U.S. Provisional Applications 60/329,508 filed October 17, 2001, 60/329,747 filed October 18, 2001 and 60/331,478 filed November 16, 2001 which are herein incorporated by reference in their entireties. The *phoA* promoter region is located at nucleotides 111-410 SEQ ID NOs:52 and 53. The MBP signal sequence is encoded by nucleotides 423-500 of SEQ ID NO:53 and nucleotides 501-959 of SEQ ID NO:53 encode amino acids 134-285 of Neutrokine-alpha (SEQ ID NO:2). The amino acid sequence of the MBP signal sequenee is shown in SEQ ID NO:54 and the amino acid sequence of the full length MBP signal sequence-neutrokine-alpha protein encoded by the pML124-MBPss-BLyS veetor is shown in SEQ ID NO:55

Neutrokine-alpha Expression in E. coli

[1055] Plasmid pML124-MBPss-BLyS was transformed into *E. coli* cells, e.g. K-12 based strains, by standard methods. Ampicillin resistant transformants were sereened for the proper DNA insert by restriction enzyme analysis and DNA sequence. For example, digestion of pML124-MBPss-BLyS™ with NdeI and KpnI results in two nucleotide fragments: 549 and 4,431 base pairs in length. Positive clones were subsequently grown in City Broth-Low Phosphate media (see recipe below). Neutrokine-alpha expression levels were examined via SDS-PAGE and subsequent Coomassie staining. Using simple shake flask experiments, more than 260 mg/L of Neutrokine-alpha was obtained.

[1056] Next, positive clones were grown to high cell density in complex media in small scale bioreactors, similar to the method described by Joly *et al.*, *PNAS* 95:2773-2777 (1998), which is hereby incorporated by reference in its entirety. Specifically, the initial fermentation medium for the 5L bioreactor was composed of 55.7 mM ammonium sulfate, 13.9 mM sodium monobasic phosphate, 21.9 mM potassium dibasic phosphate, 5 mM sodium citrate, 29.6 mM potassium chloride, 14.7 mM magnesium sulfate, 1.11%

NZ-amine AS, 1.11% yeast extract, 5 g/L glucose, 0.002% ferric chloride, 25 μg/ml kanamycin. A trace element solution (2.5 ml/3.4 L) was added containing 100 mM ferric chloride plus 30 mM of the following components: zinc sulfate, cobalt chloride, sodium molybdate, copper sulfate, boric acid and manganese sulfate. The fermenter was operated at 30°C, 650 rpm agitation, 10 standard liter/minute aeration. When the initial glucose was depleted, a concentrated glucose solution (50%) was added until the dissolved oxygen (DO) concentration reached 20% of air saturation as measured by an on-line oxygen electrode. When the optical density (600 nm) reached 40 OD600, a solution of 20% NZ amine AS, 20% yeast extract was fed at 0.2 ml/min for the rest of the fermentation. Neutrokine-alpha production was on the order of 260-570mg/L.

Low Phosphate Containing Media:

City Broth-Low Phosphate:

30mM $(NH_4)_2SO_4$; 2.25 mM NaCitrate-$2H_2O$; 12mM $MgSO_4$; 15 mM KCl; 5% Yeast extract; 2% Casamino acids; 110 mM MOPS; 33 mM Glucose; pH 7.3

Vegan City Broth-Low Phosphate:

30mM $(NH_4)_2SO_4$; 2.25 mM NaCitrate-$2H_2O$; 12mM $MgSO_4$; 15 mM KCl; 5% Phytone; 2% Casamino acids; 110 mM MOPS; 33 mM Glucose; pH 7.3

[1057] The only difference between the two media is that Phytone is substituted for Yeast extract in the Vegan reeipe.

Purification of Neutrokine-alpha

[1058] 10 grams of *E. coli* cell paste are suspended in 50 milliliters of 5mM sodium citrate, pH 6.0 and plaeed at 4°C for 1 hour with gentle shaking. Cells are then disrupted by passing them through an M-Y110 Microfluidizer® Processor (Microfluidics, Inc., Newton, MA) set at 7500 psi four times. The suspension is then centrifuged at 22,000 x *g* for twenty minutes at 4°C using a Sorvall SLA-1500 rotor. The supernatant is then collected and filtered through a 0.45 micron bottle top filter (Nalgene).

[1059] Filtered supernatant is then loaded at 9 centimeters/hour on a Fast Flow Sepharose DEAE column (Amersham Bisociences, Piscataway, NJ) previously equilibrated with 5mM sodium citrate, pH6.0 (equilibration buffer). After loading, the column is washed with 5 to 10 column volumes of equilibration buffer. The Neutrokine-alpha protein is eluted with a 200mM NaCl step in equilibration buffer. Buffers used with the Fast Flow Sepharose DEAE chromatography column are pre-filtered using a 0.22 micron CA bottle top filter (Nalgene) and pre-chilled to 4°C. The Fast Flow Sepharose DEAE column is used at 4°C. Prior to use, columns are cleaned with 0.5 M NaOH.

[1060] Relevant fractions, as determined by the ratio of contaminating proteins to Neutrokine-alpha protein seen in Coomassie stained SDS-PAGE gels, are pooled and diluted 1:1 with 10mM sodium citrate, pH 6.0, 2M $(NH_4)SO_4$. Pooled fractions are loaded at 17 centimeters/hour onto a Polypropylene Glycol Hydrophobic Interaction chromatography column (Tosoh Biosep, Montgomeryville, PA) previously equilibrated with 10mM sodium citrate, pH 6.0, 1M $(NH_4)SO_4$ (loading buffer). After loading, the column is washed with 5-10 volumes of loading buffer. The Neutrokine-alpha protein is eluted with a 5 column volume gradient from loading buffer to elution buffer (10mM sodium citrate, pH 6.0). Neutrokine-alpha elutes in the second peak toward the end of the gradient absorbance at 280nm. Buffers used with the Polypropylene Glycol Hydrophobic Interaction chromatography column are pre-filtered using a 0.22 micron CA bottle top filter (Nalgene) and used at room temperature. The Polypropylene Glycol Hydrophobic Interaction chromatography column is also used at room temperature. Prior to use, columns are cleaned with 0.5 M NaOH.

[1061] Relevant fractions, determined by the ratio of contaminating proteins to Neutrokine-alpha protein as monitored by Coomassie stained SDS-PAGE gels, are pooled and are dialyzed overnight (12 hours) into 50mM Tris, pH 7.4, 50mM NaCl at 4°C. The dialyzed pool is then loaded onto a POROS PI-50 anion exchange chromatography column (Applied Biosystems, Foster City, CA), previously equilibrated with 50mM Tris, pH 7.4, 50mM NaCl, at 17 centimeters/hour. After loading, column is washed with 5-10 volumes of loading buffer. Neutrokine-alpha is eluted using a pH step from 50mM Tris, pH 7.4, 50mM NaCl buffer to 50mM sodium citrate, pH 6.0. Relevant fractions, as determined by the ratio of contaminating proteins to Neutrokine-alpha protein seen in Coomassie stained SDS-PAGE gels, are pooled and stored at 4°C. Buffers used with the

POROS PI-50 anion exchange chromatography column are pre-filtered using a 0.22 mieron CA bottle top filter (Nalgene) and pre-chilled to 4°C. The POROS PI-50 anion exchange chromatography column is used at 4°C. Prior to use, columns are cleaned with 0.5 M NaOH.

[1062] This purifieation protocol yields 0.5-1 milligram per gram of starting cell paste based on BCA protein assay (Pierce Biotechnology, Rockford, IL) and absorbance at 280 nanometers. The protein is 96% pure as determined by reverse phase-high performance liquid chromatography (RP-HPLC). Native-PAGE and size exclusion chromatography-HPLC (SEC-HPLC) analysis indicates the protein is predominantly in trimeric form.

[1063] The production of MBPss-Neutrokine-alpha under control of the *phoA* promoter allowed more stringent, slower expression, and resulted in increased yields. In summary, the produetion of Neutrokine-alpha from the *phoA* system is scaleable and achieves 10 to 20-fold more soluble, properly folded, active material than the current system.

Example 15: Competitive Binding Studies between antibody 15C10 and 3D4.

[1064] To determine if antibodies 15C10 and 3D4 bind similar or distinct epitopes, competitive binding studies were performed.

[1065] Soluble Neutrokine-alpha (amino acids 134-284 of SEQ ID NO:2) was preincubated with 15C10 or 3D4 antibodies. Hereinafter in this example, the antibody with which Neutrokine-alpha was preincubated will be referred to as the "competing antibody". After preincubation, soluble Neutrokine-alpha-eompeting antibody complexes were captured on an ELISA plate coated with either 3D4 or 15C10. Hereinafter in this example, the antibody coated on the ELISA plate will be referred to as the "capture antibody". After binding, and wash steps, soluble Neutrokine-alpha-competing antibody complexes captured on the 3D4 or 15C10-coated ELISA plates was detected using a biotinylated polyclonal anti-Neutrokine-alpha antibody followed by a streptavidin-coupled detection agent such as horse radish peroxidase or alkaline phosphatase.

[1066] If there is no competition between the competing antibody and the capture antibody on the ELISA plate (i.e., if the two antibodies bind non-overlapping epitopes), soluble Neutrokine-alpha will be not prevented from binding to the capture antibody on

the ELISA plate and the ELISA will give a positive signal. On the other hand, if there is competition between the competing antibody and the capture antibody on the ELISA plate (i.e., if the two antibodies bind overlapping or identical epitopes), a decreased (or no) amount of soluble Neutrokine-alpha will be bound to the ELISA plate and the ELISA will give a decreased signal, compared to the signal given in the absence of competition between the two antibodies.

[1067] When an assay similar to that described above was performed using monoclonal antibodies 15C10 and 3D4, it was found that the two antibodies competed with each other, irrespective of which antibody was the competing antibody and which antibody was the capture antibody. These results indicate that 15C10 and 3D4 at least have overlapping epitopes. Isotype matched controls of irrelevant specificity (non-Neutrokine-alpha binding) were not able to compete for binding

[1068] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[1069] The entire disclosure of all publications (including patents, patent applieations, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by referenee.

[1070] Further, the Sequence Listing submitted herewith in both computer and paper forms are hereby incorporated by reference in their entireties. Additionally, the entire diselosure (ineluding the specification, sequence listing, and drawings) of each of the following U.S. Provisional and Non-Provisional Patent Applications and International Patent Applications are herein incorporated by referenee in their entireties: U.S. Provisional Application Serial Nos.: 60/368,548 filed April 1, 2002; 60/336,726 filed December 7, 2001; 60/331,478 filed November 16, 2001; 60/330,835 filed Oct 31, 2001; 60/329,747 filed October 18, 2001; and 60/329,508 filed October 17, 2001; 60/225,628 filed August 15, 2000; 60/227,008 filed August 23, 2000; 60/234,338 filed September 22, 2000; 60/240,806 filed October 17, 2000; 60/250,020 filed November 30, 2000; 60/276,248 filed March 6, 2001; 60/293,499 filed May 25, 2001; 60/296,122 filed June 7,

2001; 60/304,809 filed July, 13 2001;60/122,388 filed March 2, 1999; 60/124,097 filed March 12, 1999; 60/126,599 filed March 26, 2000; 60/127,598 filed April 2,1999; 60/130,412 filed April 16, 1999; 60/130,696 filed April 23, 1999; 60/131,278 filed April 27, 1999; 60/131,673 filed April 29, 1999; 60/136,784 filed May 28, 1999; 60/142,659 filed July 6, 1999; 60/145,824 filed July 27, 1999; 60/167,239 filed November 24, 1999; 60/168,624 filed December 3, 1999; 60/171,108 filed December 16, 1999; 60/171,626 filed December 23, 1999; 60/176,015 filed January 14, 2000; and 60/036,100 filed January 14, 1997 and U.S. Nonprovisional Application Serial Nos.:09/929,493, filed August 14, 2001; 09/588,947 filed June 8, 2000; 09/589,285 filed June 8, 2000; 09/589,286 filed June 8, 2000; 09/589,287 filed June 8, 2000; 09/589,288 filed June 8, 2000; 09/507,968 filed February 22, 2000; 09/255,794 filed February 23, 1999; and 09/005,874 filed January 12, 1998; and International Patent Application Serial Nos. PCT/US01/25549 filed August 15, 2001; PCT/US00/04336, filed February 22, 2000; and PCT/US96/17957, filed October 25, 1996.

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(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 30, paragraph 94.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 22, 1996	97768

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: 97768

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 30, paragraph 95.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
December 12, 1998	203518

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Elaine Rivers Operations - PTO Team 1 (703) 305-3678 (703) 305-3230	Authorized officer

Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. 203518

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: 203518

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent offiee without having been laid open to public inspection, the furnishing of a sample shall only be effeeted to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 30, paragraph 95.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
January 27, 2000	PTA-1158

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer - [illegible]	Authorized officer

[illegible] 1703) 305-3073 (703) 305-3230 (FAX)

Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. PTA-1158

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-1158

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the applieation.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the publie under Seetions 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the applieation has been laid open to public inspeetion (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to publie inspection, the furnishing of a sample shall only be effeeted to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reprodueed in annex Z of Volume I of the PCT Applicant's Guide). If sueh a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Offiee or any person approved by a applieant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the applieation is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 253, paragraph 519.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
January 27, 2000	PTA-1159

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer	Authorized officer

ATCC Deposit No. PTA-1159

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-1159

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a mieroorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effeeted to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the publie under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whiehever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 254, paragraph 520.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 24, 2001	PTA-3794

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. PTA-3794

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally deeided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3794

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the publie under Seetions 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the applieation has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Offiee before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 254, paragraph 520.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 24, 2001	PTA-3795

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer Elnora Rivera PCT Operations - IAP Team 1 (703) 305-3676 (703) 305-3230 (FAX)		Authorized officer	

Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. PTA-3795

CANADA

The applieant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applieant must, by a written statement, inform the International Bureau accordingly before eompletion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3795

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 415, paragraph 1052.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 8, 2001	PTA-3778

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
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Authorized officer	Authorized officer

Elinda Rivera
PCT Operations - IAPD Team 1
(703) 305-3678 (703) 305-3230 (FAX)

Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. PTA-3778

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biologieal material referred to in the application to an independent expert nominated by the Commissioner, the applieant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applieant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the publie under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a mieroorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3778

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the applieation has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the applieation has been laid open to public inspeetion (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 416, paragraph 1053.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
November 16, 2001	PTA-3867

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer: Elnora Rivera PCT Operations - (APD Team 1 (703) 305-3878 (703) 305-3230 (FAX)	Authorized officer

Revised Form PCT/RO/134 (January 2001) Petro134ep.sollist

ATCC Deposit No. PTA-3867

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3867

UNITED KINGDOM

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DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What Is Claimed Is:

1. A method of treating a cancer of the immune system comprising administering to an individual, a therapeutically effective amount of a protein comprising an amino acid sequence that is 95% or more identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of amino acid residues n to 285 of SEQ ID NO:2, where n is an integer in the range of 2-190;

(b) the amino acid sequence of amino acid residues 1 to m of SEQ ID NO:2, where m is an integer in the range of 274 to 284; and

(c) the amino acid sequence of amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 2-190 and m is an integer in the range of 274-284;

wherein the protein is radiolabeled.

2. The method of claim 1 wherein the second amino acid sequence is (a).

3. The method of claim 1 wherein the second amino acid sequence is (b).

4. The method of claim 1 wherein the second amino acid sequence is (c).

5. The method of claim 1 wherein the protein comprises an amino acid sequence that is 100% identical to the second amino acid sequence.

6. The method of claim 5 wherein the second amino acid sequence is (a).

7. The method of claim 5 wherein the second amino acid sequence is (b).

8. The method of claim 5 wherein the second amino acid sequence is (c).

9. The method of claim 1 wherein the protein also comprises a heterologous amino acid sequence.

10. The method of claim 9 wherein the heterologous amino acid sequence is the amino acid sequenee of an immunoglobulin Fc domain.

11. The method of claim 1 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

(a) ^{131}I;

(b) ^{125}I;

(e) ^{121}I;

(d) ^{112}In; and

(e) ^{99m}Tc.

12. The method of claim 11 wherein the radioistope is ^{131}I.

13. The method of elaim 1 wherein the protein is cytotoxie to Neutrokine-alpha receptor bearing cells.

14. The method of claim 1 wherein the cancer of the immune system is a tumor.

15. The method of claim 14 wherein the tumor is metastatie.

16. A method of treating a leukemia eomprising administering to an individual, a therapeutically effective amount of a protein comprising an amino aeid sequenee that is 95% or more identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequenee of amino aeid residues n to 285 of SEQ ID NO:2, where n is an integer in the range of 2-190;

(b) the amino acid sequence of amino acid residues 1 to m of SEQ ID NO:2, where m is an integer in the range of 274 to 284; and

(c) the amino aeid sequence of amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 2-190 and m is an integer in the range of 274-284;

wherein the protein is radiolabeled.

17. The method of claim 16 wherein the second amino acid sequence is (a).

18. The method of claim 16 wherein the second amino aeid sequenee is (b).

19. The method of claim 16 wherein the second amino acid sequence is (c).

20. The method of claim 16 wherein the protein comprises an amino acid sequence that is 100% identical to the second amino acid sequence.

21. The method of claim 20 wherein the second amino acid sequence is (a).

22. The method of claim 20 wherein the second amino aeid sequence is (b).

23. The method of claim 20 wherein the seeond amino aeid sequence is (c).

24. The method of claim 16 wherein the protein also comprises a heterologous amino acid sequence.

25. The method of claim 24 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

26. The method of claim 16 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

(a) ^{131}I;

(b) ^{125}I;
(c) ^{121}I;
(d) ^{112}In; and
(e) ^{99m}Tc.

27. The method of claim 26 wherein the radioistope is ^{131}I.

28. A method of treating a cancer of the immune system comprising administering to an individual, a therapeutieally effective amount of a multimeric Neutrokine-alpha protein comprising an amino acid sequence consisting of amino acids 134-285 of SEQ ID NO:2, and wherein the protein is radiolabeled.

29. The method of claim 28 wherein the protein also comprises a heterologous amino acid sequence.

30. The method of claim 29 wherein the heterologous amino aeid sequence is the amino acid sequence of an immunoglobulin Fc domain.

31. The method of elaim 28 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:
(a) ^{131}I;
(b) ^{125}I;
(c) ^{121}I;
(d) ^{112}In; and
(e) ^{99m}Tc.

32. The method of claim 31 wherein the radioistope is ^{131}I.

33. The method of claim 28 wherein the cancer of the immune system is a tumor.

34. The method of claim 33 wherein the tumor is metastatic.

35. A method of treating leukemia comprising administering to an individual, a therapeutically effective amount of a multimeric Neutrokine-alpha protein comprising an amino acid sequence consisting of amino acids 134-285 of SEQ ID NO:2 wherein the protein is radiolabeled.

36. The method of claim 35 wherein the protein also comprises a heterologous amino acid sequence.

37. The method of claim 36 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

38. The method of claim 35 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

(a) ^{131}I;
(b) ^{125}I;
(c) ^{121}I;
(d) ^{112}In; and
(e) ^{99m}Tc.

39. The method of claim 38 wherein the radioistope is ^{131}I.

40. The method of claim 35 wherein the protein is cytotoxic to Neutrokine-alpha receptor bearing cells.

41. The pML124-MBPss-BLyS plasmid.

42. A host cell transformed with the plasmid of claim 41.

43. The host cell of claim 42 which is an *E. coli* cell.

44. A method of producing neutrokine-alpha protein comprising:

(a) culturing the host cell of claim 43 under conditions sufficient to produce the Neutrokine-alpha protein encoded by the plasmid; and

(b) recovering said Neutrokine-alpha protein.

Neutrokine-α

```
1   AAATTCAGGATAACTCTCCTGAGGGGTGAGCCAAGCCCTGCCATGTAGTGCACGCAGGAC  60

61  ATCAACAAACACAGATAACAGGAAATGATCCATTCCCTGTGGTCACTTATTCTAAAGGCC  120

121 CCAACCTTCAAAGTTCAAGTAGTGATATGGATGACTCCACAGAAAGGGAGCAGTCACGCC  180
1                   M  D  D  S  T  E  R  E  Q  S  R  L              12

181 TTACTTCTTGCCTTAAGAAAAGAGAAGAAATGAAACTGAAGGAGTGTGTTTCCATCCTCC  240
13  T  S  C  L  K  K  R  E  E  M  K  L  K  E  C  V  S  I  L  P    32
                                                        CD-I

241 CACGGAAGGAAAGCCCCTCTGTCCGATCCTCCAAAGACGGAAAGCTGCTGGCTGCAACCT  300
33  R  K  E  S  P  S  V  R  S  S  K  D  G  K  L  L  A  A  T  L    52
       CD-I

301 TGCTGCTGGCACTGCTGTCTTGCTGCCTCACGGTGGTGTCTTTCTACCAGGTGGCCGCCC  360
53  L  L  A  L  L  S  C  C  L  T  V  V  S  F  Y  Q  V  A  A  L    72

361 TGCAAGGGGACCTGGCCAGCCTCCGGGCAGAGCTGCAGGGCCACCACGCGGAGAAGCTGC  420
73  Q  G  D  L  A  S  L  R  A  E  L  Q  G  H  H  A  E  K  L  P    92
          CD-II

421 CAGCAGGAGCAGGAGCCCCCAAGGCCGGCCTGGAGGAAGCTCCAGCTGTCACCGCGGGAC  480
93  A  G  A  G  A  P  K  A  G  L  E  E  A  P  A  V  T  A  G  L    112
          CD-III

                                  #
481 TGAAAATCTTTGAACCACCAGCTCCAGGAGAAGGCAACTCCAGTCAGAACAGCAGAAATA  540
113 K  I  F  E  P  P  A  P  G  E  G  N  S  S  Q  N  S  R  N  K    132

541 AGCGTGCCGTTCAGGGTCCAGAAGAAACAGTCACTCAAGACTGCTTGCAACTGATTGCAG  600
133 R  A  V  Q  G  P  E  E  T  V  T  Q  D  C  L  Q  L  I  A  D    152
                                                        CD-IV
```

FIG.1A

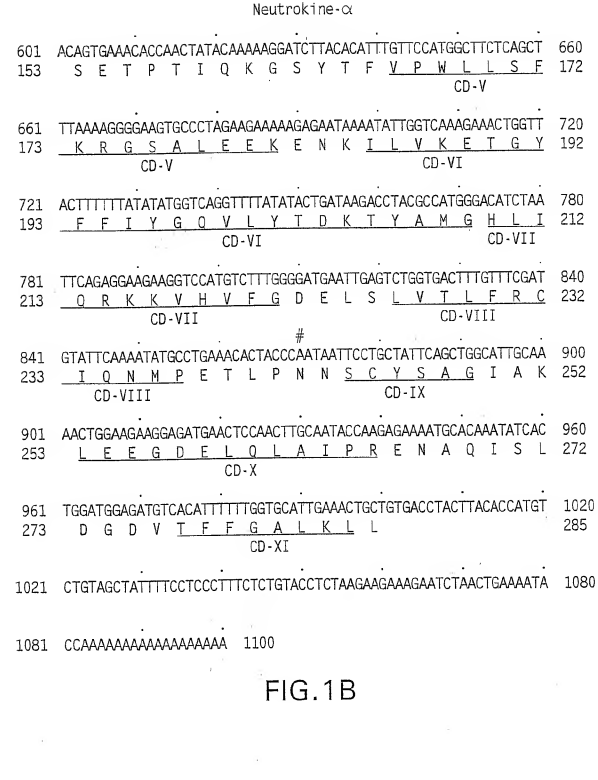

Neutrokine-α
601 ACAGTGAAACACCAACTATACAAAAAGGATCTTACACATTTGTTCCATGGCTTCTCAGCT 660
153 S E T P T I Q K G S Y T F V P W L L S F 172
CD-V
661 TTAAAAGGGGAAGTGCCCTAGAAGAAAAAGAGAATAAAATATTGGTCAAAGAAACTGGTT 720
173 K R G S A L E E K E N K I L V K E T G Y 192
CD-V CD-VI
721 ACTTTTTTATATATGGTCAGGTTTTATATACTGATAAGACCTACGCCATGGGACATCTAA 780
193 F F I Y G Q V L Y T D K T Y A M G H L I 212
CD-VI CD-VII
781 TTCAGAGGAAGAAGGTCCATGTCTTTGGGGATGAATTGAGTCTGGTGACTTTGTTTCGAT 840
213 Q R K K V H V F G D E L S L V T L F R C 232
CD-VII CD-VIII
#
841 GTATTCAAAATATGCCTGAAACACTACCCAATAATTCCTGCTATTCAGCTGGCATTGCAA 900
233 I Q N M P E T L P N N S C Y S A G I A K 252
CD-VIII CD-IX
901 AACTGGAAGAAGGAGATGAACTCCAACTTGCAATACCAAGAGAAAATGCACAAATATCAC 960
253 L E E G D E L Q L A I P R E N A Q I S L 272
CD-X
961 TGGATGGAGATGTCACATTTTTTGGTGCATTGAAACTGCTGTGACCTACTTACACCATGT 1020
273 D G D V T F F G A L K L L 285
CD-XI
1021 CTGTAGCTATTTTCCTCCCTTTCTCTGTACCTCTAAGAAGAAAGAATCTAACTGAAAATA 1080
1081 CCAAAAAAAAAAAAAAAAAA 1100

FIG.1B

10
20
30
40
50
60
70
80
90
TNFalpha
TNFbeta
LTbeta
FasLigand
Neutrokine alpha
Neutrokine alphaSV

FIG.2A

100
110
120
130
140
150
160
170
180
TNFalpha
TNFbeta
LTbeta
FasLigand
Neutrokine alpha
Neutrokine alphaSV

FIG.2B

190
200
210
220
230
240
250
260
270
TNFalpha
TNFbeta
LTbeta
FasLigand
Neutrokine alpha
Neutrokine alphaSV

FIG.2C

280
290
300
193 TNFalpha
166 TNFbeta
204 LTbeta
242 FasLigand
244 Neutrokine alpha
225 Neutrokine alphaSV
310
223 TNFalpha
196 TNFbeta
234 LTbeta
272 FasLigand
274 Neutrokine alpha
255 Neutrokine alphaSV

FIG.2D

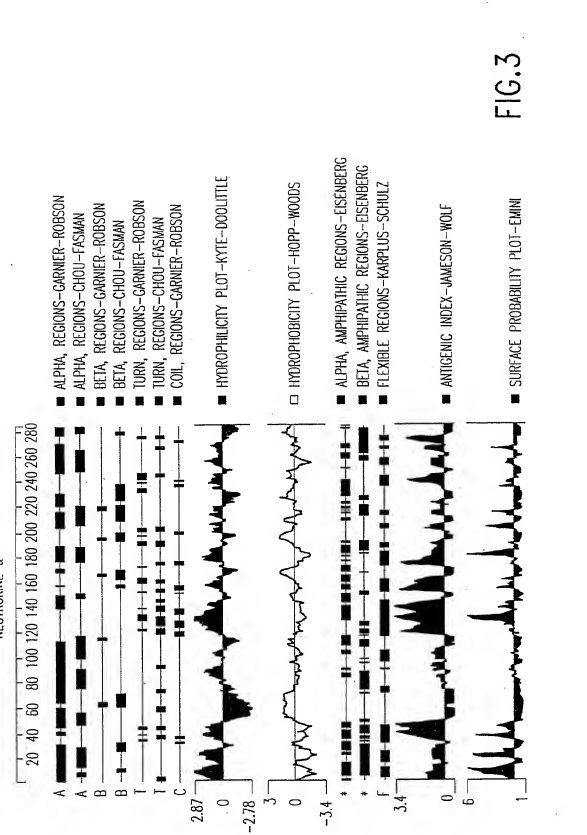

NEUTROKINE-α
20 40 60 80 100 120 140 160 180 200 220 240 260 280
A
A
B
B
T
T
C
ALPHA, REGIONS-GARNIER-ROBSON
ALPHA, REGIONS-CHOU-FASMAN
BETA, REGIONS-GARNIER-ROBSON
BETA, REGIONS-CHOU-FASMAN
TURN, REGIONS-GARNIER-ROBSON
TURN, REGIONS-CHOU-FASMAN
COIL, REGIONS-GARNIER-ROBSON
2.87
0
-2.78
HYDROPHILICITY PLOT-KYTE-DOOLITTLE
3
0
-3.4
HYDROPHOBICITY PLOT-HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS-EISENBERG
BETA, AMPHIPATHIC REGIONS-EISENBERG
FLEXIBLE REGIONS-KARPLUS-SCHULZ
3.4
0
ANTIGENIC INDEX-JAMESON-WOLF
6
1
SURFACE PROBABILITY PLOT-EMINI

FIG.3

```
            1                                                        50
HSOAD55R    .........A GGNTAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA
HNEDU15X    ...AAATTCA GGATAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA
HSLAH84R    .AATTCGGCA NAGNAAACTG GTTACTTTTT TATATATGGT CAGGTTTTAT
HLTBM08R    AATTCGGCAC GAGCAAGGCC GGCCTGGAGG AAGCTCCAGC TGTCACCGCG

            51                                                      100
HSOAD55R    GTGCACGCAG GACATCANCA A..ACACANN NNNCAGGAAA TAATCCATTC
HNEDU15X    GTGCACGCAG GACATCAACA A..ACACAGA TAACAGGAAA TGATCCATTC
HSLAH84R    ATACTGATAA GACCTACGCC ATGGGACATC TAGTTCAGAG GAAGAAGGTC
HLTBM08R    GGACTGAAAA TCTTTGAACC ACCAGCTCCA GGAGAAGGCA ACTCCAGTCA

            101                                                     150
HSOAD55R    CCTGTGGTCA CTTATTCTAA AGGCCCCAAC CTTCAAAGTT CAAGTAGTGA
HNEDU15X    CCTGTGGTCA CTTATTCTAA AGGCCCCAAC CTTCAAAGTT CAAGTAGTGA
HSLAH84R    CATGTCTTTG GGGATGAATT GAGTCTGGTG ACTTTGTTTC GATGTATTCA
HLTBM08R    GAACAGCAGA AATAAGCGTG CCGTTCAGGG TCCAGAAGAA ACAGTCACTC

            151                                                     200
HSOAD55R    TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA
HNEDU15X    TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA
HSLAH84R    AAATATGCCT GAAACACTAC CCAATAATTC CTGCTATTCA GCTGGCATTG
HLTBM08R    AAGACTGCTT GCAACTGNTT GCAGACAGTG AAACACCAAC TATACAAAAA

            201                                                     250
HSOAD55R    AGAAAAGAGA AGAAATGAAA CTGNAAGGAG TGTGTTTCCA TCCTCCCACG
HNEDU15X    AGAAAAGAGA AGAAATGAAA CT.GAAGGAG TGTGTTTCCA TCCTCCCACG
HSLAH84R    CAAAACTGGN AGGAAGGA.. ...GATGAAC TCCAACTTGC AATACCAGGG
HLTBM08R    GGCTCCCTTC TGNTGCCACA TTTGGGCCAA GGAATGGAGA GATTTCTTCG

            251                                                     300
HSOAD55R    GAAGGAAAGC CCCTCTNTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG
HNEDU15X    GAAGGAAAGC CCCTCTGTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG
HSLAH84R    GAAAATGCAC AATTATCACT GGGATGGAGA TGTTCACATT TTTTGGGTGC
HLTBM08R    TCTGGAAACA TTTTGCCAAA CTCTTCAGAT ACTCTTTNCT CTCTGGGAAT

            301                                                     350
HSOAD55R    CAACCTTGNT GNTGGCATTG TGTTCTTGCT GNCTCAAGGT GGTGTTNTTT.
HNEDU15X    CAACCTTGCT GCTGGCACTG CTGTCTTGCT GCCTCACGGT GGTGTCTTTC
HSLAH84R    CATTGAAACT GCTGTGACCT NCTTACANCA NGTGCTGTTN GCTATTTTNC
HLTBM08R    CAAAGGAAAA TCTCTACTTA GATTNACACA TTTGTTCCCA TGGGTNTCTT

            351                                                     400
HSOAD55R    ..........  ..........  ..........  ..........  ..........
HNEDU15X    TACCAGGTGG CCGCCCTGCA AGGGGACCTG GCCAGCCTCC GGGCAGAGCT
HSLAH84R    CTNCCTNTTC TNTGGTAACC TCTTAGGAAG GAAGGATTCT TAACTGGGAA
HLTBM08R    AAGTTTTAAA AGGGGAGTGC CCTTAGGAGG AAAAGGGGAT AAATATTGGC
```

FIG.4A

```
          401                                                       450
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  GCAGGGCCAC CACGCGGAGA AGCTGCCAGC AGGAGCAGGA GCCCCCAAGG
HSLAH84R  ATAACCCAAA AAAANNTTAA ANGGGTANGN GNNANANGNG GGGNNGTTNN
HLTBM08R  CAAGGNACTG GTTANTTTNT AAATATGGTC AGGTTTNTAT ANCTGGTAGG

          451                                                       500
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCGGCCTGGA GGAAGCTCCA GCTGTCACCG CGGGACTGAA AATCTTTGAA
HSLAH84R  CNNGNNGNNT TTTNGGNNTA TNTTNTNNTN GGGNNNNGTA AAAATGGGGC
HLTBM08R  CCTCGCCATG GGCATTNATT CANGGNGAGG NCNNTCTTTT GGGNTGA...

          501                                                       550
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCACCAGCTC CAGGAGAAGG CAACTCCAGT CAGAACAGCA GAAATAAGCG
HSLAH84R  CNANGGGGGN TTTTT..... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          551                                                       600
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TGCCGTTCAG GGTCCAGAAG AAACAGTCAC TCAAGACTGC TTGCAACTGA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          601                                                       650
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TTGCAGACAG TGAAACACCA ACTATACAAA AAGGATCTTA CACATTTGTT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          651                                                       700
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCATGGCTTC TCAGCTTTAA AAGGGGAAGT GCCCTAGAAG AAAAAGAGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          701                                                       750
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TAAAATATTG GTCAAAGAAA CTGGTTACTT TTTTATATAT GGTCAGGTTT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          751                                                       800
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TATATACTGA TAAGACCTAC GCCATGGGAC ATCTAATTCA GAGGAAGAAG
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........
```

FIG.4B

```
                801                                                      850
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  GTCCATGTCT TTGGGGATGA ATTGAGTCTG GTGACTTTGT TTCGATGTAT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

                851                                                      900
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TCAAAATATG CCTGAAACAC TACCCAATAA TTCCTGCTAT TCAGCTGGCA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

                901                                                      950
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TTGCAAAACT GGAAGAAGGA GATGAACTCC AACTTGCAAT ACCAAGAGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

                951                                                     1000
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  AATGCACAAA TATCACTGGA TGGAGATGTC ACATTTTTTG GTGCATTGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

               1001                                                     1050
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  ACTGCTGTGA CCTACTTACA CCATGTCTGT AGCTATTTTC CTCCCTTTCT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

               1051                                                     1100
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CTGTACCTCT AAGAAGAAAG AATCTAACTG AAAATACCAA AAAAAAAAAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

               1101
HSOAD55R  ......
HNEDU15X  AAAAAA
HSLAH84R  ......
HLTBM08R  ......
```

FIG.4C

Neutrokine-αSV

```
  1 ATGGATGACTCCACAGAAAGGGAGCAGTCACGCCTTACTTCTTGCCTTAAGAAAAGAGAA  60
  1 M  D  D  S  T  E  R  E  Q  S  R  L  T  S  C  L  K  K  R  E   20

 61 GAAATGAAACTGAAGGAGTGTGTTTCCATCCTCCCACGGAAGGAAAGCCCCTCTGTCCGA 120
 21 E  M  K  L  K  E  C  V  S  I  L  P  R  K  E  S  P  S  V  R   40
                                   CD-I

121 TCCTCCAAAGACGGAAAGCTGCTGGCTGCAACCTTGCTGCTGGCACTGCTGTCTTGCTGC 180
 41 S  S  K  D  G  K  L  L  A  A  T  L  L  L  A  L  L  S  C  C   60
       CD-I

181 CTCACGGTGGTGTCTTTCTACCAGGTGGCCGCCCTGCAAGGGGACCTGGCCAGCCTCCGG 240
 61 L  T  V  V  S  F  Y  Q  V  A  A  L  Q  G  D  L  A  S  L  R   80
                                         CD-II

241 GCAGAGCTGCAGGGCCACCACGCGGAGAAGCTGCCAGCAGGAGCAGGAGCCCCCAAGGCC 300
 81 A  E  L  Q  G  H  H  A  E  K  L  P  A  G  A  G  A  P  K  A  100
       CD-II                                CD-III

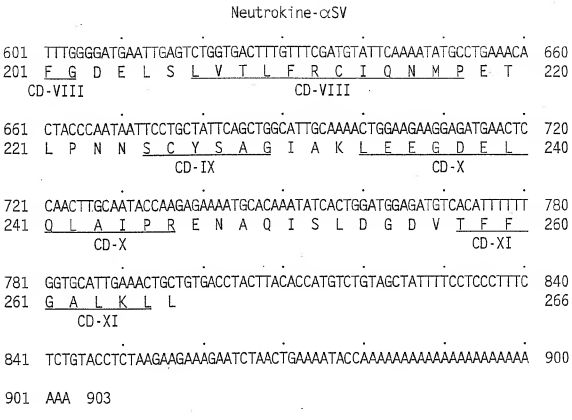
301 GGCCTGGAGGAAGCTCCAGCTGTCACCGCGGGACTGAAAATCTTTGAACCACCAGCTCCA 360
101 G  L  E  E  A  P  A  V  T  A  G  L  K  I  F  E  P  P  A  P  120
    CD-III
          #
361 GGAGAAGGCAACTCCAGTCAGAACAGCAGAAATAAGCGTGCCGTTCAGGGTCCAGAAGAA 420
121 G  E  G  N  S  S  Q  N  S  R  N  K  R  A  V  Q  G  P  E  E  140

421 ACAGGATCTTACACATTTGTTCCATGGCTTCTCAGCTTTAAAAGGGGAAGTGCCCTAGAA 480
141 T  G  S  Y  T  F  V  P  W  L  L  S  F  K  R  G  S  A  L  E  160
                                      CD-IV

481 GAAAAAGAGAATAAAATATTGGTCAAAGAAACTGGTTACTTTTTTATATATGGTCAGGTT 540
161 E  K  E  N  K  I  L  V  K  E  T  G  Y  F  F  I  Y  G  Q  V  180
    CD-IV                          CD-V

541 TTATATACTGATAAGACCTACGCCATGGGACATCTAATTCAGAGGAAGAAGGTCCATGTC 600
181 L  Y  T  D  K  T  Y  A  M  G  H  L  I  Q  R  K  K  V  H  V  200
             CD-VI                          CD-VII
```

FIG.5A


Neutrokine-αSV
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201 F G D E L S L V T L F R C I Q N M P E T 220
CD-VIII
CD-VIII
661 CTACCCAATAATTCCTGCTATTCAGCTGGCATTGCAAAACTGGAAGAAGGAGATGAACTC 720
221 L P N N S C Y S A G I A K L E E G D E L 240
CD-IX
CD-X
721 CAACTTGCAATACCAAGAGAAAATGCACAAATATCACTGGATGGAGATGTCACATTTTTT 780
241 Q L A I P R E N A Q I S L D G D V T F F 260
CD-X
CD-XI
781 GGTGCATTGAAACTGCTGTGACCTACTTACACCATGTCTGTAGCTATTTTCCTCCCTTTC 840
261 G A L K L L 266
CD-XI
841 TCTGTACCTCTAAGAAGAAAGAATCTAACTGAAAATACCAAAAAAAAAAAAAAAAAAAAA 900
901 AAA 903

FIG.5B

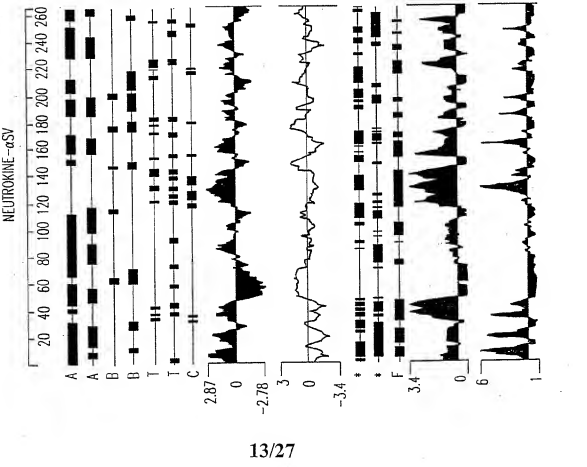
NEUTROKINE-αSV
20 40 60 80 100 120 140 160 180 200 220 240 260
A
A
B
B
T
T
C
2.87
0
-2.78
3
0
-3.4
*
*
F
3.4
0
6
1
ALPHA, REGIONS-GARNIER-ROBSON
ALPHA, REGIONS-CHOU-FASMAN
BETA, REGIONS-GARNIER-ROBSON
BETA, REGIONS-CHOU-FASMAN
TURN, REGIONS-GARNIER-ROBSON
TURN, REGIONS-CHOU-FASMAN
COIL, REGIONS-GARNIER-ROBSON
HYDROPHILICITY PLOT-KYTE-DOOLITTLE
HYDROPHOBICITY PLOT-HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS-EISENBERG
BETA, AMPHIPATHIC REGIONS-EISENBERG
FLEXIBLE REGIONS-KARPLUS-SCHULZ
ANTIGENIC INDEX-JAMESON-WOLF
SURFACE PROBABILITY PLOT-EMINI

FIG.6

Neutrokine-
Alpha
Transmembrane Region
A
A'
B'
B
C
D
E
April
TNF
LT α

FIG. 7A-1

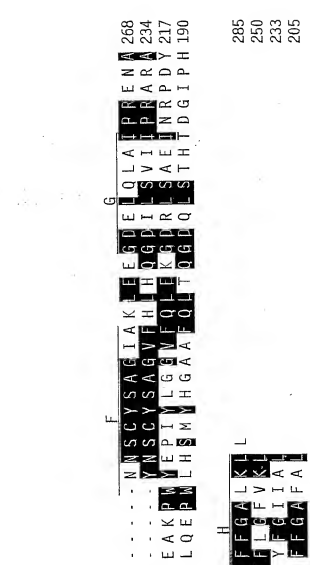
E--TLD-----NNSCYSAGIAKLEEGDELQLAIPRENA 268
SHPDRA-----YNSCYSAGVFHLHQGDIISVIIPRARA 234
CQRETPEGAEAKPWEPIYLGGVFQLEKGDRLSAELNRPDY 217
YP----GLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIPH 190
QISIDGDVIFFGALKLL 285
KLNISCHGTLLSFVKLI 250
LDFAESGQVYFGIIAFI 233
LVLS-STVFFGAFAIL 205

FIG.7A-2

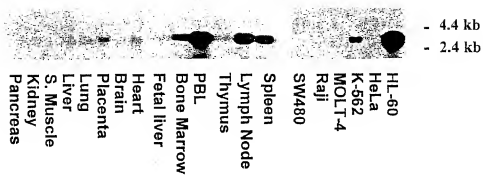
- 4.4 kb
- 2.4 kb
HL-60
HeLa
K-562
MOLT-4
Raji
SW480
Spleen
Lymph Node
Thymus
PBL
Bone Marrow
Fetal liver
Heart
Brain
Placenta
Lung
Liver
S. Muscle
Kidney
Pancreas

FIG.7B

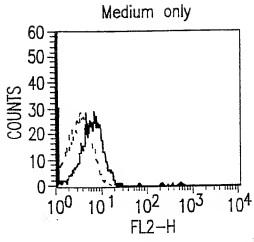

FIG.8A

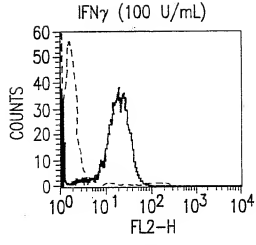

FIG.8B

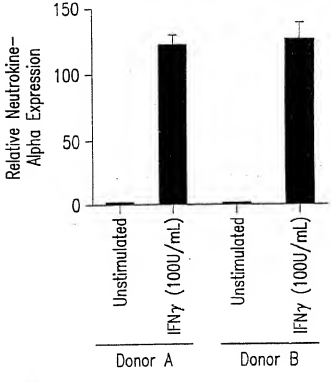

FIG.8C

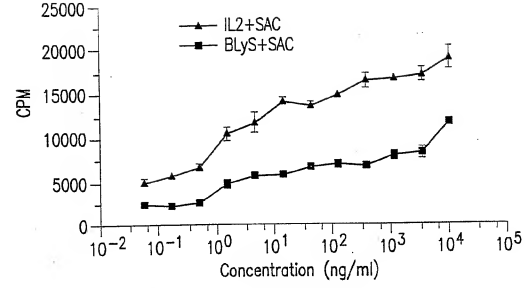
IL2+SAC
BLyS+SAC
CPM
25000
20000
15000
10000
5000
0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Concentration (ng/ml)

FIG. 9A

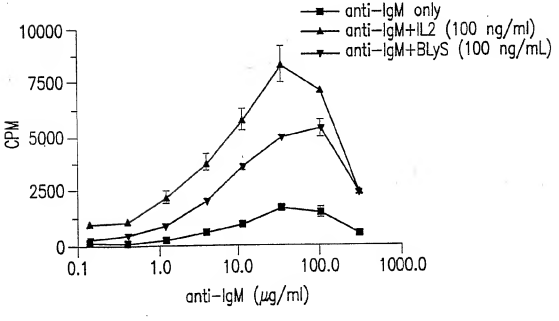
anti-IgM only
anti-IgM+IL2 (100 ng/ml)
anti-IgM+BLyS (100 ng/mL)
CPM
10000
7500
5000
2500
0
0.1
1.0
10.0
100.0
1000.0
anti-IgM (μg/ml)

FIG. 9B

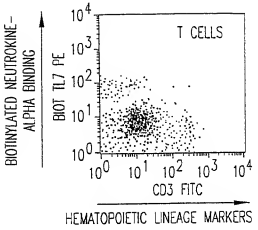

FIG.10A

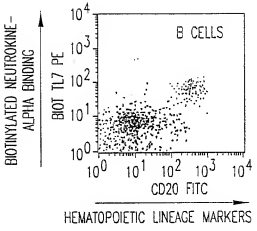

FIG.10B

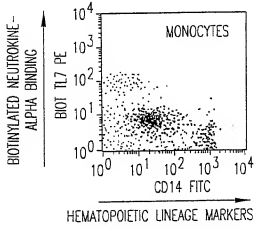

FIG.10C

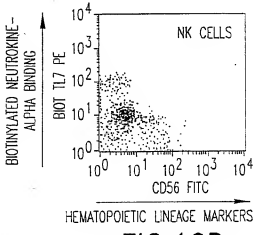

FIG.10D

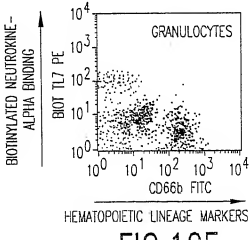

FIG.10E

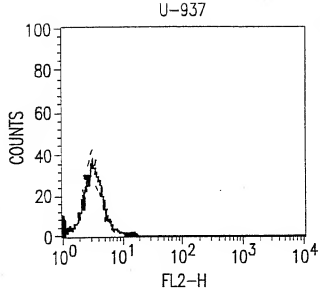

FIG.10F

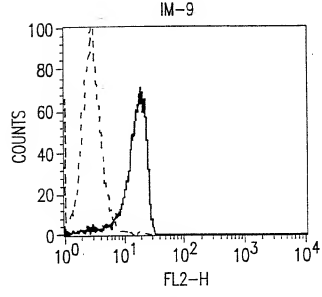

FIG.10G

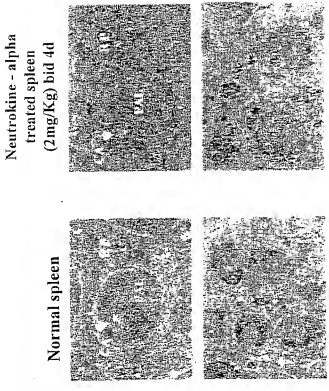
Normal spleen
Neutrokine - alpha treated spleen (2mg/Kg) bid 4d
H & E (100X)
CD45R(B220) (40X)

FIG.11A

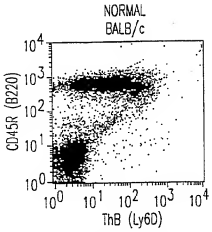

FIG. 11B

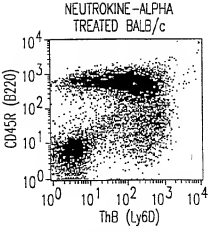

FIG. 11C

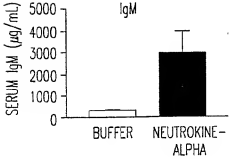

FIG. 11D

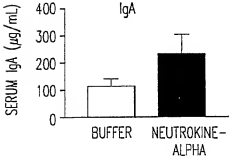

FIG. 11E

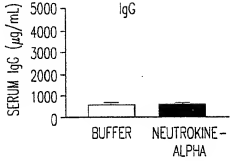

FIG. 11F

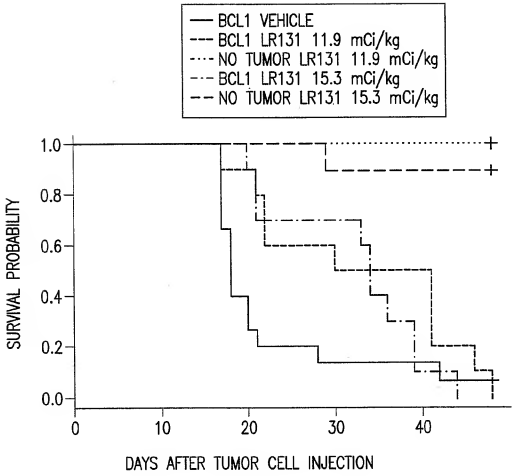
BCL1 VEHICLE
BCL1 LR131 11.9 mCi/kg
NO TUMOR LR131 11.9 mCi/kg
BCL1 LR131 15.3 mCi/kg
NO TUMOR LR131 15.3 mCi/kg
SURVIVAL PROBABILITY
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
DAYS AFTER TUMOR CELL INJECTION

FIG.12

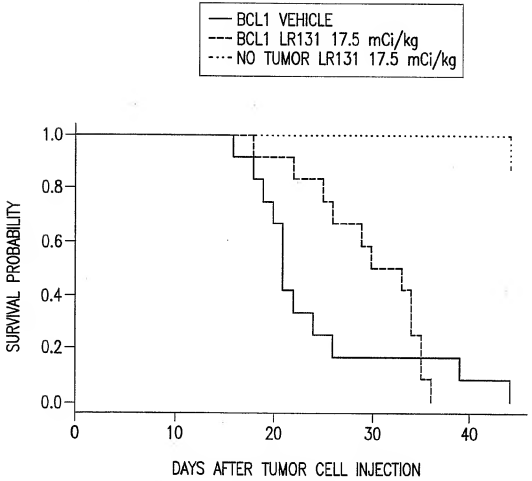
BCL1 VEHICLE
BCL1 LR131 17.5 mCi/kg
NO TUMOR LR131 17.5 mCi/kg
SURVIVAL PROBABILITY
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
DAYS AFTER TUMOR CELL INJECTION

FIG.13

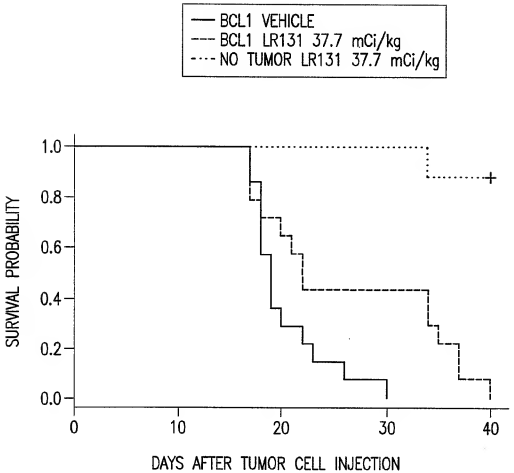
BCL1 VEHICLE
BCL1 LR131 37.7 mCi/kg
NO TUMOR LR131 37.7 mCi/kg
SURVIVAL PROBABILITY
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
DAYS AFTER TUMOR CELL INJECTION

FIG.14

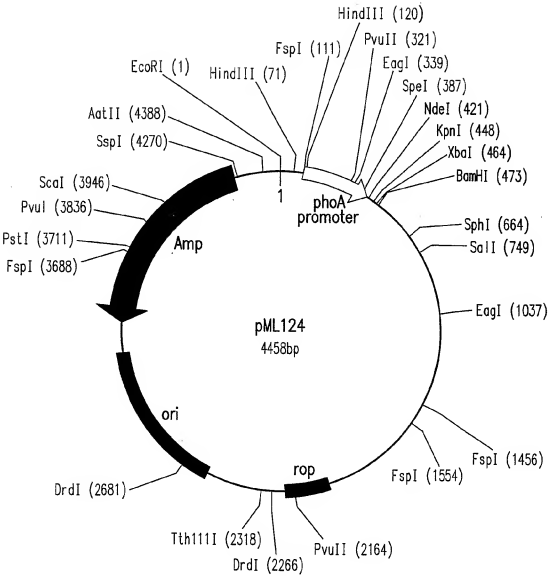
HindIII (120)
PvuII (321)
EagI (339)
SpeI (387)
NdeI (421)
KpnI (448)
XbaI (464)
BamHI (473)
FspI (111)
HindIII (71)
EcoRI (1)
AatII (4388)
SspI (4270)
ScaI (3946)
PvuI (3836)
PstI (3711)
FspI (3688)
1
phoA promoter
SphI (664)
SalI (749)
Amp
EagI (1037)
pML124
4458bp
ori
rop
FspI (1456)
FspI (1554)
DrdI (2681)
Tth111I (2318)
DrdI (2266)
PvuII (2164)

FIG.15

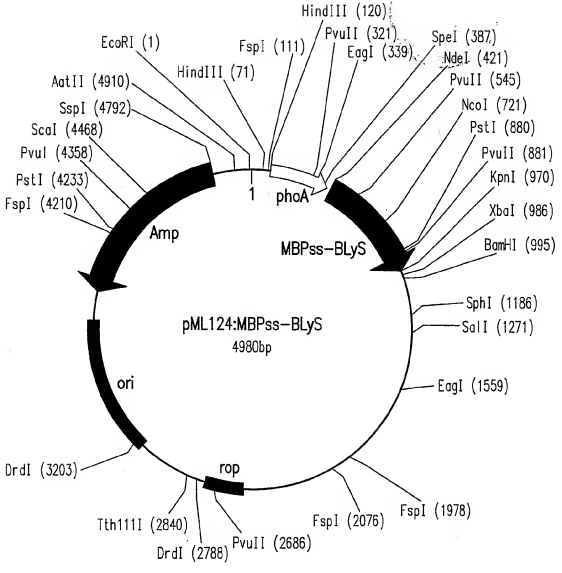
HindIII (120)
PvuII (321)
EagI (339)
SpeI (387)
NdeI (421)
PvuII (545)
NcoI (721)
PstI (880)
PvuII (881)
KpnI (970)
XbaI (986)
BamHI (995)
SphI (1186)
SalI (1271)
EagI (1559)
FspI (1978)
FspI (2076)
PvuII (2686)
DrdI (2788)
Tth111I (2840)
DrdI (3203)
EcoRI (1)
FspI (111)
HindIII (71)
AatII (4910)
SspI (4792)
ScaI (4468)
PvuI (4358)
PstI (4233)
FspI (4210)
1
phoA
Amp
MBPss-BLyS
pML124:MBPss-BLyS
4980bp
ori
rop

FIG.16

SEQUENCE LISTING

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<130> PF343PCT4

<140> Unassigned
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<150> 60/368,548
<151> 2002-04-01

<150> 60/336,726
<151> 2001-12-07

<150> 60/331,478
<151> 2001-11-16

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<151> 2001-10-31

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<170> PatentIn Ver. 3.1

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<222> (147)..(1001)

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atcaacaaac acagataaca ggaaatgatc cattccctgt ggtcacttat tctaaaggcc 120

ccaaccttca aagttcaagt agtgat atg gat gac tcc aca gaa agg gag cag 173
Met Asp Asp Ser Thr Glu Arg Glu Gln
1 5

tca cgc ctt act tct tgc ctt aag aaa aga gaa gaa atg aaa ctg aag 221
Ser Arg Leu Thr Ser Cys Leu Lys Lys Arg Glu Glu Met Lys Leu Lys
10 15 20 25

gag tgt gtt tcc atc ctc cca cgg aag gaa agc ccc tct gtc cga tcc 269
Glu Cys Val Ser Ile Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser
30 35 40

tcc aaa gac gga aag ctg ctg gct gca acc ttg ctg ctg gca ctg ctg 317

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Ser Lys Asp Gly Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu
                45                  50                  55

tct tgc tgc ctc acg gtg gtg tct ttc tac cag gtg gcc gcc ctg caa   365
Ser Cys Cys Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln
        60                  65                  70

ggg gac ctg gcc agc ctc cgg gca gag ctg cag ggc cac cac gcg gag   413
Gly Asp Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu
    75                  80                  85

aag ctg cca gca gga gca gga gcc ccc aag gcc ggc ctg gag gaa gct   461
Lys Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala
 90                  95                 100                 105

cca gct gtc acc gcg gga ctg aaa atc ttt gaa cca cca gct cca gga   509
Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro Gly
                110                 115                 120

gaa ggc aac tcc agt cag aac agc aga aat aag cgt gcc gtt cag ggt   557

Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln Gly
            125                 130                 135

cca gaa gaa aca gtc act caa gac tgc ttg caa ctg att gca gac agt   605
Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser
        140                 145                 150

gaa aca cca act ata caa aaa gga tct tac aca ttt gtt cca tgg ctt   653
Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu
    155                 160                 165

ctc agc ttt aaa agg gga agt gcc cta gaa gaa aaa gag aat aaa ata   701
Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile
170                 175                 180                 185

ttg gtc aaa gaa act ggt tac ttt ttt ata tat ggt cag gtt tta tat   749
Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr
                190                 195                 200

act gat aag acc tac gcc atg gga cat cta att cag agg aag aag gtc   797
Thr Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val
            205                 210                 215

cat gtc ttt ggg gat gaa ttg agt ctg gtg act ttg ttt cga tgt att   845
His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile
        220                 225                 230

caa aat atg cct gaa aca cta ccc aat aat tcc tgc tat tca gct ggc   893
Gln Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly
    235                 240                 245

att gca aaa ctg gaa gaa gga gat gaa ctc caa ctt gca ata cca aga   941
Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg
250                 255                 260                 265

gaa aat gca caa ata tca ctg gat gga gat gtc aca ttt ttt ggt gca   989
Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala
                270                 275                 280
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ttg aaa ctg ctg tgacctactt acaccatgtc tgtagctatt ttcctccctt       1041
Leu Lys Leu Leu
        285
tctctgtacc tctaagaaga aagaatctaa ctgaaaatac caaaaaaaaa aaaaaaaaa  1100


<210> 2
<211> 285
<212> PRT
<213> Homo sapiens

<400> 2
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
  1               5                  10                  15

Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
             20                  25                  30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
         35                  40                  45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
     50                  55                  60

Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65                  70                  75                  80

Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                 85                  90                  95

Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110

Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
        115                 120                 125

Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
    130                 135                 140

Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
145                 150                 155                 160

Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
                165                 170                 175

Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
            180                 185                 190

Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
        195                 200                 205

Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
    210                 215                 220

Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
225                 230                 235                 240

Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                245                 250                 255
```

```
Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
            260                 265                 270

Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
        275                 280                 285



<210> 3
<211> 233
<212> PRT
<213> Homo sapiens

<400> 3
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
  1               5                  10                  15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20                  25                  30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50                  55                  60


Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
65                  70                  75                  80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
                85                  90                  95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
            100                 105                 110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
        115                 120                 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
    130                 135                 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145                 150                 155                 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                165                 170                 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
            180                 185                 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
        195                 200                 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
    210                 215                 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225                 230
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<210> 4
<211> 205
<212> PRT
<213> Homo sapiens

<400> 4
Met Thr Pro Pro Glu Arg Leu Phe Leu Pro Arg Val Arg Gly Thr Thr
1 5 10 15

Leu His Leu Leu Leu Leu Gly Leu Leu Leu Val Leu Leu Pro Gly Ala
20 25 30

Gln Gly Leu Pro Gly Val Gly Leu Thr Pro Ser Ala Ala Gln Thr Ala
35 40 45

Arg Gln His Pro Lys Met His Leu Ala His Ser Thr Leu Lys Pro Ala
50 55 60

Ala His Leu Ile Gly Asp Pro Ser Lys Gln Asn Ser Leu Leu Trp Arg
65 70 75 80

Ala Asn Thr Asp Arg Ala Phe Leu Gln Asp Gly Phe Ser Leu Ser Asn
85 90 95

Asn Ser Leu Leu Val Pro Thr Ser Gly Ile Tyr Phe Val Tyr Ser Gln
100 105 110

Val Val Phe Ser Gly Lys Ala Tyr Ser Pro Lys Ala Thr Ser Ser Pro

115 120 125

Leu Tyr Leu Ala His Glu Val Gln Leu Phe Ser Ser Gln Tyr Pro Phe
130 135 140

His Val Pro Leu Leu Ser Ser Gln Lys Met Val Tyr Pro Gly Leu Gln
145 150 155 160

Glu Pro Trp Leu His Ser Met Tyr His Gly Ala Ala Phe Gln Leu Thr
165 170 175

Gln Gly Asp Gln Leu Ser Thr His Thr Asp Gly Ile Pro His Leu Val
180 185 190

Leu Ser Pro Ser Thr Val Phe Phe Gly Ala Phe Ala Leu
195 200 205

<210> 5
<211> 244
<212> PRT
<213> Homo sapiens

<400> 5
Met Gly Ala Leu Gly Leu Glu Gly Arg Gly Gly Arg Leu Gln Gly Arg
1 5 10 15

Gly Ser Leu Leu Leu Ala Val Ala Gly Ala Thr Ser Leu Val Thr Leu
20 25 30

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Leu Leu Ala Val Pro Ile Thr Val Leu Ala Val Leu Ala Leu Val Pro
          35                  40                  45

Gln Asp Gln Gly Gly Leu Val Thr Glu Thr Ala Asp Pro Gly Ala Gln
      50                  55                  60

Ala Gln Gln Gly Leu Gly Phe Gln Lys Leu Pro Glu Glu Glu Pro Glu
 65                  70                  75                  80

Thr Asp Leu Ser Pro Gly Leu Pro Ala Ala His Leu Ile Gly Ala Pro
                 85                  90                  95

Leu Lys Gly Gln Gly Leu Gly Trp Glu Thr Thr Lys Glu Gln Ala Phe
            100                 105                 110

Leu Thr Ser Gly Thr Gln Phe Ser Asp Ala Glu Gly Leu Ala Leu Pro
        115                 120                 125

Gln Asp Gly Leu Tyr Tyr Leu Tyr Cys Leu Val Gly Tyr Arg Gly Arg
    130                 135                 140

Ala Pro Pro Gly Gly Gly Asp Pro Gln Gly Arg Ser Val Thr Leu Arg
145                 150                 155                 160

Ser Ser Leu Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly Thr Pro Glu
                165                 170                 175

Leu Leu Leu Glu Gly Ala Glu Thr Val Thr Pro Val Leu Asp Pro Ala
            180                 185                 190

Arg Arg Gln Gly Tyr Gly Pro Leu Trp Tyr Thr Ser Val Gly Phe Gly
        195                 200                 205

Gly Leu Val Gln Leu Arg Arg Gly Glu Arg Val Tyr Val Asn Ile Ser
    210                 215                 220

His Pro Asp Met Val Asp Phe Ala Arg Gly Lys Thr Phe Phe Gly Ala
225                 230                 235                 240

Val Met Val Gly
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<210> 6
<211> 281
<212> PRT
<213> Homo sapiens

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<400> 6
Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp
  1               5                  10                  15

Ser Ser Ala Ser Ser Pro Trp Ala Pro Pro Gly Thr Val Leu Pro Cys
            20                  25                  30

Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro
        35                  40                  45

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro
```

```
     50                  55                  60

Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly Asn His Ser Thr Gly
 65                  70                  75                  80

Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly
                85                  90                  95

Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala
            100                 105                 110

Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu
        115                 120                 125

Lys Gln Ile Gly His Pro Ser Pro Pro Pro Glu Lys Lys Glu Leu Arg
    130                 135                 140

Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu
145                 150                 155                 160

Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr
                165                 170                 175

Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr
            180                 185                 190

Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser
        195                 200                 205

His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met
    210                 215                 220

Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala
225                 230                 235                 240

Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His
                245                 250                 255

Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser
            260                 265                 270

Gln Thr Phe Phe Gly Leu Tyr Lys Leu
        275                 280
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<210> 7
<211> 338
<212> DNA
<213> Homo sapiens

<220>
<223> Description of Combined DNA/RNA Molecule: n equals a, t, g, or c

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<222> (3)
<223> n equals a, t, g, or c

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<223> n equals a, t, g or c

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<223> n equals a, t, g, or c

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aaacacannn nncaggaaat aatccattcc ctgtggtcac ttattctaaa ggccccaacc 120

ttcaaagttc aagtagtgat atggatgact ccacagaaag ggagcagtca cgccttactt 180

cttgccttaa gaaaagagaa gaaatgaaac tgnaaggagt gtgtttccat cctcccacgg 240

aaggaaagcc cctctntccg atcctccaaa gacggaaagc tgctggctgc aaccttgntg 300

ntggcattgt gttcttgctg nctcaaggtg gtgttntt 338

<210> 8
<211> 509
<212> DNA
<213> Homo sapiens

<220>
<223> Description of Combined DNA/RNA Molecule: n equals
a, t, g, or c

<220>
<221> misc_feature
<222> (10)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (13)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (209)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (315)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (322)
<223> n equals a, t, g, or c

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<222> (325)
<223> n equals a, t, g, or c

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<220>
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<223> n equals a, t, g, or c

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<222> (416)
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<222> (424)
<223> n equals a, t, g, or c

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<222> (426)..(427)
<223> n equals a, t, g, or c

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<222> (433)
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<220>
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<223> n equals a, t, g, or c

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<222> (458)
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<222> (461)..(462)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (466)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (469)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (471)..(472)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (474)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (478)..(481)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
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<220>
<221> misc_feature

<222> (498)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (504)
<223> n equals a, t, g, or c

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aattcggcan agnaaactgg ttacttttttt atatatggtc aggttttata tactgataag 60
acctacgcca tgggacatct agttcagagg aagaaggtcc atgtctttgg ggatgaattg 120
agtctggtga ctttgtttcg atgtattcaa aatatgcctg aaacactacc caataattcc 180

tgctattcag ctggcattgc aaaactggna ggaaggagat gaactccaac ttgcaatacc 240
aggggaaaat gcacaattat cactgggatg gagatgttca catttttttgg gtgccattga 300
aactgctgtg acctncttac ancangtgct gttngctatt ttncctncct nttctntggt 360
aacctcttag gaaggaagga ttcttaactg ggaaataacc caaaaaaann ttaaangggt 420
angngnnana ngnggggnng ttnncnngnn gnnttttngg nntatnttnt nntngggnnn 480
ngtaaaaatg gggccnangg gggnttttt 509

<210> 9
<211> 497
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (168)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (213)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (288)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (325)
<223> n equals a, t, g, or c

<220>
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<222> (346)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (406)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (415)
<223> n equals a, t, g, or c

<220>
<221> misc_feature

<222> (419)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (437)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (442)
<223> n equals a, t, g, or c

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<221> misc_feature
<222> (467)
<223> n equals a, t, g, or c

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<222> (473)
<223> n equals a, t, g, or c

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<222> (476)
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<220>
<221> misc_feature
<222> (481)
<223> n equals a, t, g, or c

<220>
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<222> (483)..(484)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (494)
<223> n equals a, t, g, or c

<400> 9
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tctttgaacc accagctcca ggagaaggca actccagtca gaacagcaga aataagcgtg 120
ccgttcaggg tccagaagaa acagtcactc aagactgctt gcaactgntt gcagacagtg 180
aaacaccaac tatacaaaaa ggctcccttc tgntgccaca tttgggccaa ggaatggaga 240
gatttcttcg tctggaaaca ttttgccaaa ctcttcagat actctttnct ctctgggaat 300
caaaggaaaa tctctactta gattnacaca tttgttccca tgggtntctt aagttttaaa 360
aggggagtgc ccttaggagg aaaaggggat aaatattggc caaggnactg gttantttnt 420
aaatatggtc aggtttntat anctggtagg cctcgccatg ggcattnatt canggngagg 480
ncnntctttt gggntga 497

<210> 10
<211> 27
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha forward primer containing BamHI

restriction site

<400> 10
gtgggatcca gcctccgggc agagctg 27

<210> 11
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha reverse primer containing HindIII restriction site and sequence complementary to two stop codons

<400> 11
gtgaagcttt tattacagca gtttcaatgc acc 33

<210> 12
<211> 26
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha forward primer containing BspHI restriction site

<400> 12
gtgtcatgag cctccgggca gagctg 26

<210> 13
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha reverse primer containing HindIII restriction site and sequence complementary to two stop codons

<400> 13
gtgaagcttt tattacagca gtttcaatgc acc 33

<210> 14
<211> 28
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha forward primer containing BamHI restriction site

<400> 14
gtgggatccc cgggcagagc tgcagggc 28

<210> 15

<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha reverse primer containing BamHI
restriction site and sequence complementary to two
stop codons

<400> 15
gtgggatcct tattacagca gtttcaatgc acc 33

<210> 16
<211> 129
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha forward primer containing a Bam HI
restriction site, Kozak sequence, AUG start codon,
and sequence encoding the secretory leader peptide of
human IL-6 gene

<400> 16
gcgggatccg ccaccatgaa ctccttctcc acaagcgcct tcggtccagt tgccttctcc 60
ctggggctgc tcctggtgtt gcctgctgcc ttccctgccc cagttgtgag acaaggggac 120
ctggccagc 129

<210> 17
<211> 30
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha reverse primerconating BamHI
restriction site

<400> 17
gtgggatcct tacagcagtt tcaatgcacc 30

<210> 18
<211> 903
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(798)

<400> 18
atg gat gac tcc aca gaa agg gag cag tca cgc ctt act tct tgc ctt 48
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
1 5 10 15

aag aaa aga gaa gaa atg aaa ctg aag gag tgt gtt tcc atc ctc cca 96
Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
20 25 30

cgg aag gaa agc ccc tct gtc cga tcc tcc aaa gac gga aag ctg ctg 144

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Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
          35                  40                  45

gct gca acc ttg ctg ctg gca ctg ctg tct tgc tgc ctc acg gtg gtg   192
Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
     50                  55                  60

tct ttc tac cag gtg gcc gcc ctg caa ggg gac ctg gcc agc ctc cgg   240
Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65                  70                  75                  80

gca gag ctg cag ggc cac cac gcg gag aag ctg cca gca gga gca gga   288
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                 85                  90                  95

gcc ccc aag gcc ggc ctg gag gaa gct cca gct gtc acc gcg gga ctg   336
Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110

aaa atc ttt gaa cca cca gct cca gga gaa ggc aac tcc agt cag aac   384
Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
        115                 120                 125

agc aga aat aag cgt gcc gtt cag ggt cca gaa gaa aca gga tct tac   432
Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Gly Ser Tyr
    130                 135                 140

aca ttt gtt cca tgg ctt ctc agc ttt aaa agg gga agt gcc cta gaa   480
Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu
145                 150                 155                 160

gaa aaa gag aat aaa ata ttg gtc aaa gaa act ggt tac ttt ttt ata   528
Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
                165                 170                 175

tat ggt cag gtt tta tat act gat aag acc tac gcc atg gga cat cta   576
Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu
            180                 185                 190

att cag agg aag aag gtc cat gtc ttt ggg gat gaa ttg agt ctg gtg   624
Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val
        195                 200                 205

act ttg ttt cga tgt att caa aat atg cct gaa aca cta ccc aat aat   672
Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn
    210                 215                 220

tcc tgc tat tca gct ggc att gca aaa ctg gaa gaa gga gat gaa ctc   720
Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu
225                 230                 235                 240

caa ctt gca ata cca aga gaa aat gca caa ata tca ctg gat gga gat   768
Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp
                245                 250                 255

gtc aca ttt ttt ggt gca ttg aaa ctg ctg tgacctactt acaccatgtc     818
Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
            260                 265

tgtagctatt ttcctccctt tctctgtacc tctaagaaga aagaatctaa ctgaaaatac 878
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caaaaaaaaa aaaaaaaaaa aaaaa 903

<210> 19
<211> 266
<212> PRT
<213> Homo sapiens

<400> 19
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
1 5 10 15

Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
20 25 30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
35 40 45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
50 55 60

Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
65 70 75 80

Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
85 90 95

Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
100 105 110

Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
115 120 125

Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Gly Ser Tyr
130 135 140

Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu
145 150 155 160

Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
165 170 175

Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu
180 185 190

Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val
195 200 205

Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn
210 215 220

Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu
225 230 235 240

Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp
245 250 255

Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
260 265

<210> 20
<211> 136

<212> PRT
<213> Homo sapiens

<400> 20
His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys Asp Asp
1 5 10 15

Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg Gly Arg
20 25 30

Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala Gly Val
35 40 45

Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe Thr Met
50 55 60

Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr Leu Phe
65 70 75 80

Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr Asn Ser
85 90 95

Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile Leu Ser
100 105 110

Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro His Gly
115 120 125

Thr Phe Leu Gly Phe Val Lys Leu
130 135

<210> 21
<211> 462
<212> DNA
<213> Homo sapiens

<400> 21
atggctgttc agggtccgga agaaaccgtt actcaggact gccttcagct gatcgcagac 60
tctgaaactc cgaccatcca gaaaggttct tacacctttg ttccttggct gctttctttc 120
aaacgtggtt ctgccctgga agagaaagaa aacaaaatcc tggttaaaga aactggttac 180
ttctttatct acggtcaggt tctttacact gataagacct acgccatggg tcacctgatt 240
cagcgtaaga aagttcacgt tttcggtgac gagctgtctc tggttactct gtttcgctgc 300
attcagaaca tgccggaaac tcttcctaac aactcctgct actctgctgg catcgcaaaa 360
ctggaagagg gtgatgaact gcagctggca attcctcgtg aaaacgcaca aatttctctg 420
gacggtgatg taaccttctt tggtgcactg aaacttctgt aa 462

<210> 22
<211> 1040
<212> DNA
<213> Mus musculus

<220>

<221> CDS
<222> (1)..(468)

<400> 22
cgc gtg gta gac ctc tca gct cct cct gca cca tgc ctg cct gga tgc 48
Arg Val Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro Gly Cys
1 5 10 15

cgc cat tct caa cat gat gat aat gga atg aac ctc aga aac aga act 96
Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn Arg Thr
20 25 30

tac aca ttt gtt cca tgg ctt ctc agc ttt aaa aga gga aat gcc ttg 144
Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu
35 40 45

gag gag aaa gag aac aaa ata gtg gtg agg caa aca ggc tat ttc ttc 192
Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe
50 55 60

atc tac agc cag gtt cta tac acg gac ccc atc ttt gct atg ggt cat 240
Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His
65 70 75 80

gtc atc cag agg aag aaa gta cac gtc ttt ggg gac gag ctg agc ctg 288
Val Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
85 90 95

gtg acc ctg ttc cga tgt att cag aat atg ccc aaa aca ctg ccc aac 336
Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn
100 105 110

aat tcc tgc tac tcg gct ggc atc gcg agg ctg gaa gaa gga gat gag 384
Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu
115 120 125

att cag ctt gca att cct cgg gag aat gca cag att tca cgc aac gga 432
Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly
130 135 140

gac gac acc ttc ttt ggt gcc cta aaa ctg ctg taa ctcacttgct 478
Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
145 150 155

ggagtgcgtg atccccttcc ctcgtcttct ctgtacctcc gagggagaaa cagacgactg 538

gaaaaactaa aagatgggga aagccgtcag cgaaagtttt ctcgtgaccc gttgaatctg 598

atccaaacca ggaaatataa cagacagcca caaccgaagt gtgccatgtg agttatgaga 658

aacggagccc gcgctcagaa agaccggatg aggaagaccg ttttctccag tcctttgcca 718

acacgcaccg caaccttgct ttttgccttg ggtgacacat gttcagaatg cagggagatt 778

tccttgtttt gcgatttgcc atgagaagag ggcccacaac tgcaggtcac tgaagcattc 838

acgctaagtc tcaggattta ctctcccttc tcatgctaag tacacacacg ctcttttcca 898

ggtaatacta tgggatacta tggaaaggtt gtttgttttt aaatctagaa gtcttgaact 958

ggcaatagac aaaaatcctt ataaattcaa gtgtaaaata aacttaatta aaaaggttta 1018

agtgtgaaaa aaaaaaaaa aa 1040

<210> 23
<211> 155
<212> PRT
<213> Mus musculus

<400> 23
Arg Val Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro Gly Cys
1 5 10 15
Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn Arg Thr
20 25 30
Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu
35 40 45
Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe
50 55 60
Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His
65 70 75 80
Val Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
85 90 95
Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn
100 105 110
Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu
115 120 125
Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly
130 135 140
Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
145 150 155

<210> 24
<211> 26
<212> DNA
<213> Homo sapiens

<400> 24
ccaccagctc caggagaagg caactc 26

<210> 25
<211> 19
<212> DNA
<213> Homo sapiens

<400> 25
accgcgggac tgaaaatct 19

<210> 26
<211> 23
<212> DNA
<213> Homo sapiens

<400> 26

cacgcttatt tctgctgttc tga 23

<210> 27
<211> 657
<212> DNA
<213> Macaca irus

<400> 27
taccaggtgg cggccgtgca aggggacctg gccagcctcc gggcagagct gcagggccac 60
cacgcggaga agctgccagc aagagcaaga gcccccaagg ccggtctggg ggaagctcca 120
gctgtcaccg caggactgaa aatctttgaa ccaccagctc caggagaagg caactccagt 180
cagagcagca gaaataagcg tgctattcag ggtgcagaag aaacagtcat tcaagactgc 240
ttgcaactga ttgcagacag tgaaacacca actatacaaa aaggatctta cacatttgtt 300
ccatggcttc tcagctttaa aaggggaagt gccctagaag aaaaagagaa taaaatattg 360
gtcaaagaaa ctggttactt ttttatatat ggtcaggttt tatacactga taagacctat 420
gccatgggac atctaattca gaggaaaaaa gtccatgtct ttggggatga attgagtctg 480
gtgactttgt ttcgatgtat tcaaaatatg cctgaaacac tacccaataa ttcctgctat 540
tcagctggca ttgcaaaact ggaagaagga gatgaacttc aacttgcaat accacgagaa 600
aatgcacaaa tatcactgga tggagatgtc acatttttg gtgccctcaa actgctg 657

<210> 28
<211> 219
<212> PRT
<213> Macaca irus

<400> 28
Tyr Gln Val Ala Ala Val Gln Gly Asp Leu Ala Ser Leu Arg Ala Glu
1 5 10 15

Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Arg Ala Arg Ala Pro
20 25 30

Lys Ala Gly Leu Gly Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile
35 40 45

Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Ser Ser Arg
50 55 60

Asn Lys Arg Ala Ile Gln Gly Ala Glu Glu Thr Val Ile Gln Asp Cys
65 70 75 80

Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser
85 90 95

Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu
100 105 110

Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe
115 120 125

Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
130 135 140

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
145 150 155 160

Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn
165 170 175

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Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu
            180                 185                 190

Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly
        195                 200                 205

Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
    210                 215
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<210> 29
<211> 657
<212> DNA
<213> Macaca mulatta (Rhesus Monkey)

<400> 29
taccaggtgg cggccgtgca aggggacctg gccagcctcc gggcagagct gcagagccac 60
cacgcggaga agctgccagc aagagcaaga gccccaagg ccggtctggg ggaagctcca 120
gctgtcaccg cgggactgaa aatctttgaa ccaccagctc caggagaagg caactccagt 180
cagagcagca gaaataagcg tgctattcag ggtgcagaag aaacagtcat tcaagactgc 240
ttgcaactga ttgcagacag tgaaacacca actatacaaa aaggatctta cacatttgtt 300
ccatggcttc tcagctttaa aaggggaagt gccctagaag aaaaagagaa taaaatattg 360
gtcaaagaaa ctggttactt ttttatatat ggtcaggttt tatacactga taagacctat 420
gccatgggac atctaattca gaggaaaaaa gtccatgtct ttggggatga attgagtctg 480
gtgactttgt ttcgatgtat tcaaaatatg cctgaaacac tacccaataa ttcctgctat 540
tcagctggca ttgcaaaact ggaagaaggg gatgaacttc aacttgcaat accacgagaa 600
aatgcacaaa tatcactgga tggagatgtc acatttttttg gtgccctcaa actgctg 657

<210> 30
<211> 219
<212> PRT
<213> Macaca mulatta (Rhesus Monkey)

<400> 30
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Tyr Gln Val Ala Ala Val Gln Gly Asp Leu Ala Ser Leu Arg Ala Glu
  1               5                  10                  15

Leu Gln Ser His His Ala Glu Lys Leu Pro Ala Arg Ala Arg Ala Pro
             20                  25                  30

Lys Ala Gly Leu Gly Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile
         35                  40                  45

Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Ser Ser Arg
     50                  55                  60

Asn Lys Arg Ala Ile Gln Gly Ala Glu Glu Thr Val Ile Gln Asp Cys
 65                  70                  75                  80

Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser
                 85                  90                  95

Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu
            100                 105                 110

Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe
        115                 120                 125
```

```
Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
    130                 135                 140

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
145                 150                 155                 160

Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn
                165                 170                 175

Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu
            180                 185                 190

Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly
        195                 200                 205

Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
    210                 215


<210> 31
<211> 38
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokine-alpha forward primer containing sequence
      encoding PSC signal peptide C-terminus

<400> 31
ggtcgccgtt tctaacgcgg ccgttcaggg tccagaag                          38


<210> 32
<211> 49
<212> DNA
<213> Homo sapiens

<220>
<223> Reverse primer for amplifying Neutrokine-alpha containing
      reverse complement sequence of the pA2GP vectorand Kpn I
      restriction site

<400> 32
ctggttcggc ccaaggtacc aagcttgtac cttagatctt ttctagatc               49


<210> 33
<211> 21
<212> DNA
<213> Homo sapiens

<220>
<223> forward primer that anneals to PSC baculovirus transfer
      plasmid pMGS12

<400> 33
ctggtagttc ttcggagtgt g                                            21
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<210> 34
<211> 19
<212> DNA
<213> Homo sapiens

<220>
<223> reverse primer that anneals to PSC baculovirus transfer plasmid pMGS12

<400> 34
cgcgttagaa acggcgacc 19

<210> 35
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (7)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (12)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (16)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<223> Neutrokine-alpha degenerate oligonucleotide forward primer

<400> 35
taccagntgg cngccntgca ag 22

<210> 36
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (14)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (16)..(17)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<223> Neutrokine-alpha degenerate oligonucleotide reverse primer

<400> 36
gtnacagcag tttnanngca cc 22

<210> 37
<211> 867
<212> DNA
<213> Mus musculus

<400> 37
atggatgagt ctgcaaagac cctgccacca ccgtgcctct gtttttgctc cgagaaagga 60
gaagatatga aagtgggata tgatcccatc actccgcaga aggaggagcg tgcctggttt 120
gggatctgca gggatggaag gctgctggct gctacccttcc tgctggccct gttgtccagc 180
agtttcacag cgatgtcctt gtaccagttg gctgccttgc aagcagacct gatgaacctg 240
cgcatggagc tgcagagcta ccgaggttca gcaacaccag ccgccgcggg tgctccagag 300
ttgaccgctg gagtcaaact cctgacaccg gcagctcctc gaccccacaa ctccagccgc 360
ggccacagga acagacgcgc cttccaggga ccagaggaaa cagaacaaga tgtagacctc 420
tcagctcctc ctgcaccatg cctgcctgga tgccgccatt ctcaacatga tgatsatgga 480
atgaacctca gaaacatcat tcaagactgt ctgcagctga ttgcagacag cgacacgccg 540
gccttggagg agaaagagaa caaaatagtg gtgaggcaaa caggctattt cttcatctac 600
agccaggttc tatacacgga ccccatcttt gctatgggtc atgtcatcca gaggaagaaa 660

gtacacgtct ttggggacga gctgagcctg gtgaccctgt tccgatgtat tcagaatatg 720
cccaaaacac tgcccaacaa ttcctgctac tcggctggca tcgcgaggct ggaagaagga 780
gatgagattc agcttgcaat tcctcgggag aatgcacaga tttcacgcaa cggagacgac 840
accttctttg gtgccctaaa actgctg 867

<210> 38
<211> 289
<212> PRT
<213> Mus musculus

<400> 38
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
1 5 10 15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
20 25 30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
35 40 45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
50 55 60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65 70 75 80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
85 90 95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
100 105 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe

```
        115                 120                 125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
    130                 135                 140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
145                 150                 155                 160

Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
                165                 170                 175

Ser Asp Thr Pro Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg
            180                 185                 190

Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro
        195                 200                 205

Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val Phe
    210                 215                 220

Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met
225                 230                 235                 240

Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg
                245                 250                 255

Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala
            260                 265                 270

Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu
        275                 280                 285

Leu
```

<210> 39
<211> 309
<212> PRT
<213> Mus musculus

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<400> 39
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
  1               5                  10                  15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
            20                  25                  30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
        35                  40                  45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
    50                  55                  60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65                  70                  75                  80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
                85                  90                  95
```

```
Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
            100                 105                 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
        115                 120                 125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
    130                 135                 140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
145                 150                 155                 160

Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
                165                 170                 175

Ser Asp Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp
            180                 185                 190

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
        195                 200                 205

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
    210                 215                 220

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
225                 230                 235                 240

Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
                245                 250                 255

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala

            260                 265                 270

Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
        275                 280                 285

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
    290                 295                 300

Ala Leu Lys Leu Leu
305


<210> 40
<211> 290
<212> PRT
<213> Mus musculus

<400> 40
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
  1               5                  10                  15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
            20                  25                  30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
        35                  40                  45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
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```
     50                  55                  60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
 65                  70                  75                  80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
                85                  90                  95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
            100                 105                 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
        115                 120                 125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
    130                 135                 140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
145                 150                 155                 160

Met Asn Leu Arg Asn Arg Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser
                165                 170                 175

Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val
            180                 185                 190

Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp
        195                 200                 205

Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val
    210                 215                 220

Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn
225                 230                 235                 240

Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala
                245                 250                 255

Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn
            260                 265                 270

Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys
        275                 280                 285

Leu Leu
    290


<210> 41
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<212> PRT
<213> Rattus sp

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Ile Ala Asp Ser Asn Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe
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Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys
         35                  40                  45

Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser
     50                  55                  60

Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln
 65                  70                  75                  80

Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu
                 85                  90                  95

Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys
            100                 105                 110

Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Val Gln Leu
        115                 120                 125

Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr
    130                 135                 140

Phe Phe Gly Ala Leu Lys Leu Leu
145                 150


<210> 42
<211> 165
<212> PRT
<213> Rattus sp

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Thr Pro Val Pro Ser Leu Pro Gly Asn Cys His Ala Ser His His Asp
             20                  25                  30


Glu Asn Gly Leu Asn Leu Arg Thr Arg Thr Tyr Thr Phe Val Pro Trp
         35                  40                  45

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
     50                  55                  60

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
 65                  70                  75                  80

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
                 85                  90                  95

Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
            100                 105                 110

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
        115                 120                 125

Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
    130                 135                 140

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
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Ala Leu Lys Leu Leu
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<210> 43
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<212> PRT
<213> Rattus sp

<400> 43
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Thr Pro Ala Pro Ser Leu Pro Gly Asn Cys His Ala Ser His His Asp
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Ile Ala Asp Ser Asn Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe
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Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys
65 70 75 80

Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser
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Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln
100 105 110

Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu
115 120 125

Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys
130 135 140

Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu

145 150 155 160

Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr
165 170 175

Phe Phe Gly Ala Leu Lys Leu Leu
180

<210> 44
<211> 133
<212> PRT
<213> Rattus sp

<400> 44
Ala Phe Gln Gly Pro Glu Glu Thr Gly Thr Tyr Thr Phe Val Pro Trp
1 5 10 15

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
20 25 30

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Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
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Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
     50                  55                  60

Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
 65                  70                  75                  80

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
                85                  90                  95

Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
            100                 105                 110

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
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Ala Leu Lys Leu Leu
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<213> Homo sapiens

<400> 45

Met Leu Gln Asn Ser Ala Val Leu Leu Leu Leu Val Ile Ser Ala Ser
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<223> consensus signal sequence

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<213> Homo sapiens

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Leu Ser Trp Gly Ala Ala Leu Gly Ala Val Ala Cys Ala Met Ala Leu
        35                  40                  45

Leu Thr Gln Gln Thr Glu Leu Gln Ser Leu Arg Arg Glu Val Ser Arg
    50                  55                  60

Leu Gln Gly Thr Gly Gly Pro Ser Gln Asn Gly Glu Gly Tyr Pro Trp
65                  70                  75                  80

Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala Leu Glu Ala Trp Glu Asn
                85                  90                  95

Gly Glu Arg Ser Arg Lys Arg Arg Ala Val Leu Thr Gln Lys Gln Lys
            100                 105                 110

Lys Gln His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys
        115                 120                 125

Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg
    130                 135                 140

Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala
145                 150                 155                 160

Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe
                165                 170                 175

Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr
            180                 185                 190

Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr
        195                 200                 205

Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile
    210                 215                 220

Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro
225                 230                 235                 240

His Gly Thr Phe Leu Gly Phe Val Lys Leu

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<210> 49
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide Primer

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<210> 50
<211> 21
<212> DNA
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<220>
<223> Oligonucleotide Primer

<400> 50
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<210> 51
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> Oligonucleotide primer

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<210> 52
<211> 4458
<212> DNA
<213> Artificial

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<213> Artificial Sequence

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<223> MBP signal sequence

<400> 54
Met Lys Ile Lys Thr Gly Ala Arg Ile Leu Ala Leu Ser Ala Leu Thr
1 5 10 15

Thr Met Met Phe Ser Ala Ser Ala Leu Ala
20 25

<210> 55
<211> 178
<212> PRT
<213> Artificial Sequence

<220>
<223> MBPss-Neutrokine-alpha

<400> 55
Met Lys Ile Lys Thr Gly Ala Arg Ile Leu Ala Leu Ser Ala Leu Thr
1 5 10 15

Thr Met Met Phe Ser Ala Ser Ala Leu Ala Ala Val Gln Gly Pro Glu
20 25 30

Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr
35 40 45

Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser
50 55 60

Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val
65 70 75 80

Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp
85 90 95

Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His Val
100 105 110

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Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn
        115                 120                 125

Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala
    130                 135                 140

Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn
145                 150                 155                 160

Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys
                165                 170                 175

Leu Leu
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<210> 56
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> wild-type Shine Dalgarno box and its adjacent sequence

<400> 56
tttgtacatg gagaaaataa a 21

<210> 57
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> optimized Shine Dalgarno box and its adjacent sequence

<400> 57
cacgtaaagg aagtatctca t 21